THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXV JANUARY, 1949 NUMBER 1

PURE GRANULOMATOUS NOCARDIOSIS: A NEW FUNGUS DISEASE DISTINGUISHED BY INTRACELLULAR PARASITISM

A DESCRIPTION OF A NEW DISEASE IN MAN DUE TO A HITHERTO UNDESCRIBED ORGANISM, NOCARDIA INTRACELLULARIS, N. SP., INCLUDING A STUDY OF THE BIOLOGIC AND PATHOGENIC PROPERTIES OF THIS SPECIES*

JOHN T. CUTTINO, M.D., and ANNE M. MCCABE, A.B.

(From the Department of Pathology, Duke University School of Medicine, Durham, N.C.)

Phagocytosis by reticulo-endothelial cells, together with proliferation of these cells, resulting from the presence of an offending agent, has been regarded generally as the fundamental basis of granulomatous inflammation. The majority of these reactions quickly become complicated by necrosis, fibrosis, alteration in structure of the macrophage, and appearance of other cellular elements such as lymphocytes and plasma cells. In human disease one rarely encounters a granulomatous reaction consisting entirely of phagocytosis and proliferation of macrophages. This is most nearly approached in the lesions of typhoid fever, histoplasmosis, and early stages of lymphogranuloma inguinale, but even here complicating features are to be found.

Opportunity was afforded recently to study a human reaction which assumed the form of phagocytosis with reticulo-endothelial cell proliferation and minimal necrosis. The agent which induced this reaction is an acid-fast organism which may be isolated easily in culture.

REPORT OF CASE

The patient was a white girl, 34 months old, whose illness began with anorexia, nausea, vomiting, and progressive weight loss about 4½ months before her death. Shortly after the onset a mass, thought to be a lymphosarcoma, was noted in the abdomen. The urine was reported as negative; hemoglobin, 8.4 gm. (52 per cent); red blood cells, 3,100,000; white blood cells, 20,150 (polymorphonuclear leukocytes, 33 per cent; eosinophils, 3 per cent; lymphocytes, 62 per cent; monocytes, 2 per cent). A transfusion of 225 cc. of type A blood and five treatments of deep x-ray therapy (dosage not known) were administered. Because of unfavorable reaction,

* Presented at the Forty-Fourth Annual Meeting of The American Association of Pathologists and Bacteriologists, Chicago, May 16, 1947.

Received for publication, October 1, 1947.

radiotherapy was discontinued. She then was admitted to Duke Hospital for an exploratory operation.

Physical Examination. The child was emaciated; her skin was pale and dry. The lymph nodes of the axillary, cervical, and inguinal groups were enlarged. The chest was clear to percussion and auscultation; the heart showed no abnormality. Pulse was 120; blood pressure, 88/50 mm. Hg. In the abdomen, prominent in the upper quadrants, was a large, irregular mass. There was diminished muscular tone, especially of the extremities and abdomen.

Course. Shortly after admission an enlarged inguinal node was removed for study. The node showed complete alteration of its architecture with proliferation of the macrophages (Fig. 1). The cytoplasm of these macrophages appeared foamy and granular. Inasmuch as they resembled Gaucher's cells, a lipodystrophy was considered. However, by the use of Ziehl-Neelsen stain it was demonstrated that these cells contained massive numbers of an acid-fast bacilliform organism. A second biopsy showed a lesion of essentially the same type, and the organism was isolated in pure culture. Before the first biopsy was completed, the patient had received five x-ray treatments of 50 r., but upon the discovery of acid-fast organisms radiotherapy was discontinued. She then was treated by supportive therapy with intravenous supplemental proteins (amigen) and by chemotherapy, specifically, sodium p,-p'-diaminodiphenylsulfone-N,N' didextrose sulfonate (promin) and penicillin. The entire hospital course was afebrile except for two minor elevations of temperature to 38.5°C. and 38.8°C., respectively. The blood counts fluctuated: Hemoglobin, 8.1 gm.; red blood cells, 4.2 millions; white blood cells, between 7,500 and 19,360, with a differential formula of 26 per cent polymorphonuclear leukocytes, 38 per cent staff cells, 19 per cent juveniles, 4.5 per cent lymphocytes, and 12.5 per cent monocytes. Serologic tests for syphilis were negative. Total plasma proteins were 3.6 gm. per 100 cc.; albumin, 1.3 gm. per 100 cc.; globulin, 2.3 gm. per 100 cc.; albumin/globulin ratio, 0.56; cholesterol, 100 to 110. About 2 months before death the stools contained 30.49 per cent fat, with no parasites or ova; but many acid-fast organisms were found by both smears and culture. Other laboratory data were noncontributory.

On proctoscopic examination irregular ulcers were observed. A biopsy of one of these ulcers showed proliferation of macrophages containing many acid-fast organisms. Examinations of nasal smears for acid-fast organisms were negative. Intradermal tests with old tuberculin and avian tuberculin gave negative results, although the patient's serum was said to have agglutinated a suspension of her own organisms in a dilution of 1:640. The patient grew progressively weaker despite supportive measures, and died on her 78th hospital day, 4½ months after the onset of her illness.

AUTOPSY FINDINGS

Gross Examination

The body was emaciated, with accentuation of bony prominences. The *abdomen* was conspicuously distended. When the *abdominal cavity* was opened, approximately 500 cc. of chylous fluid escaped. Similar fluid was found in lesser quantity (150 cc.) in both *pleural cavities*. The *lacteal vessels* on the surface of the intestines were congested. The abdominal organs were displaced by a large, space-consuming mass in the mesentery and retroperitoneal regions (Fig. 2). This mass consisted of greatly enlarged and matted *lymph nodes*, which

on section were brilliant yellow. In the central portion of this mass were scattered, irregular zones of necrosis. The *pancreas, kidneys,* and *adrenals* were imbedded in the mass but not infiltrated by it, and were not otherwise abnormal. The *small intestine* showed no lesions, but ulcers occurred throughout the length of the *colon* (Fig. 3). These ulcers were eroded and indurated, with overhanging edges. Their bases were covered by blood-stained, greenish, necrotic material. The *lymph nodes* near the intestine were enlarged and bright yellow. The *spleen* was only slightly enlarged, and scattered throughout were yellowish areas which were interpreted grossly as malpighian corpuscles. The *liver* showed a prominent architecture with no focal lesions. The *mediastinal lymph nodes* were slightly enlarged. The *heart* and *lungs* were without gross lesions. The *brain* appeared normal. The *bone marrow* of the sternum, vertebra, and femur was pale, but gross lesions were not recognized.

Microscopic Examination

The architecture of the *spleen* was greatly distorted by an abundant proliferation of large, pale, foamy-appearing macrophages to the extent that lymphoid remnants appeared only as focal collections of cells (Fig. 4). The malpighian corpuscles were replaced by epithelioid cells, concentrically arranged about the central artery (Fig. 8). These structures were delineated by what appeared to be collapsed sinusoids containing small quantities of blood. Lymphocytes were virtually absent, although a few scattered plasma cells could be found. There was no conglomeration of these structures, despite their juxtaposition. The epithelioid cells contained numerous intracellular acid-fast organisms (Fig. 10), simulating the parasitism found in leprosy and Johne's disease. There were a few isolated organisms of varying lengths in the interstices. Branching forms were not observed. Groups of multinucleated giant cells, usually in clusters of a dozen or more, occurred throughout the spleen. The nuclei were in a peripheral position, and, for the most part, were small and hyperchromatic; only a few of the pale, vesicular type were found. In the central zone of the cytoplasm there was a clear, spherical, homogeneous area (Figs. 9 and 11) which proved to be devoid of organisms, but the foamy, granular cytoplasm around it contained acid-fast organisms, usually radially arranged. Foot's silver preparations failed to demonstrate reticulum, and even with Masson's trichrome stain little fibrosis was recognized.

The architectural pattern of the *lymph nodes* was as distorted as that of the spleen (Fig. 5), and there were similar groups of large giant cells

(Figs. 8 and 11). The epithelioid pattern found in the spleen was nowhere apparent in the nodes. In some zones the multinucleated giant cells were so numerous as to form broad sheets. Isolated areas of necrosis were found in the retro-aortic and mesenteric lymph nodes. Only a few of these areas were large. Elsewhere necrosis was not a feature of the lesion. Several recanalized thrombi in fairly large arteries were noted.

The floor of the *ulcers in the colon* consisted of fibrous scar tissue, with intermingled groups of macrophages containing very numerous acid-fast organisms. There were remarkably few leukocytes and plasma cells, commonly seen in intestinal ulcers. Groups of macrophages with phagocytized organisms were noted also in nonulcerated areas, interpreted as replacement of lymphoid follicles by reticulo-endothelial cells. They frequently interrupted, by infiltration, the muscularis mucosae. The ulcers were thought to arise by surface erosion of the masses of proliferating macrophages.

Scattered tubercle-like nodules of epithelioid cells occurred in the *liver* (Fig. 6). The epithelioid cells contained many acid-fast organisms (Fig. 7). Many Kupffer cells throughout the liver showed phagocytosis of the organism, but there was no apparent damage to liver cells. In routine preparations of the *lung* no lesions were found. However, by acid-fast technics, organisms were demonstrated in the submucosal lymphoid follicles of the bronchi and in perivascular macrophages. Here again, massive intracellular parasitism was apparent.

In the mucosa of the *appendix* the lymphoid element was somewhat proliferative, and here also were many intracellular acid-fast organisms; but no tuberculoid arrangement was seen. The tissue about the *kidneys, pancreas,* and *adrenals* contained foci of epithelioid proliferation and there was intracellular parasitism. However, lesions were not found within the parenchyma save for scattered calcium deposits in the medullary tubules of the kidney.

In sections of the *myocardium,* calcific subendocardial deposits were found in the vicinity of the mitral valve, and there was a mild vacuolization of myocardial fibers, but no granuloma.

Microscopic studies of the *bladder, uterus, ovary, diaphragm, thyroid, pituitary body,* and *brain* were noncontributory. These organs appeared normal; no aggregates of organisms were found.

Anatomic Diagnoses. A peculiar form of granuloma characterized by intracellular parasitism and proliferation of macrophages producing complete replacement of lymphoid tissue of mesenteric, retroperitoneal, mediastinal, and left subclavian lymph nodes, and spleen; partial re-

placement of bone marrow and lymphoid tissue of gastro-intestinal tract; and microscopic proliferation of macrophages with intracellular organisms in liver and lungs; granulomatous ulceration of colon; congestion of lacteal lymphatics, chylous ascites (500 cc.), bilateral pleural effusion (150 cc.); calcium deposition in subendocardial tissue and renal tubules; and emaciation.

INTERPRETATION

The outstanding feature of the disease in this case was the peculiar host-parasite relationship. The sole local reaction appeared to be a stimulation of phagocytic activity of the macrophages of the reticulo-endothelial system with proliferation of these phagocytes. As a consequence, the predominant lesions occurred in the spleen, lymph nodes, and bone marrow. This feature was emphasized further by the fact that in the small lymphoid collections of the bronchi, in the liver, and around the vessels in the pancreas was found marked phagocytosis of these organisms by large macrophages. This process could not be appreciated in ordinary preparations and was recognized only when the tissue was stained by the acid-fast technic.

This host-parasite relationship was characterized further by the predominantly intracellular position of the parasite. However, in a few areas, particularly in the lymph nodes and the spleen, extracellular organisms were noted; but with the impressively dominant intracellular parasitism organisms in the interstices were interpreted as having "spilled over" from the macrophages. The organisms appeared to be in equilibrium with the cells and thus were capable of growth and multiplication. The formation of giant cells, with peripherally placed nuclei and a clear central zone, was interpreted as support for the concept of Doan, Sabin, and Forkner [1] regarding the formation of giant cells of the Langhans' type by amitotic division of macrophages. By supravital staining these authors have shown that the peripheral disposition of the nuclei in Langhans' giant cells is due to the centrally placed "rosettes of fine vacuoles," which in the living state are stainable with neutral red. It seems likely, therefore, that the clear central zones seen in many of the giant cells in the lymph nodes and in the spleen of this case were due to the presence of such "rosettes."

This host-parasite relationship seemed to be characterized further by the fact that no product of metabolism of the organism was toxic to its host. Such a point of view seems substantiated by the fact that throughout her illness the patient did not exhibit a consistent febrile response. In addition, there was no leukotaxis, in that none of the

lesions showed significant numbers of polymorphonuclear leukocytes or other elements of suppurative inflammation. In this connection it is noteworthy that early in her illness there was an absolute lymphocytosis. However, following x-ray therapy and progress of the disease a degenerative shift of the hemogram occurred. Necrosis was not a conspicuous feature. There were zones of necrosis in the large masses of lymph nodes of the mesenteric and retroperitoneal groups, but the more discrete nodes and the spleen did not show this feature. It may be pointed out that the large mass in the abdomen had received a total dosage of approximately 500 r. of deep x-ray therapy. In addition, two large vessels with old recanalized thrombi were found in these nodes at autopsy.

Intracellular parasitism by an acid-fast organism, as presented here, finds its analogue in human leprosy, Johne's disease in cattle, and rat leprosy. However, in human leprosy there are elements in the process of inflammation other than those which characterize the disease under consideration, and the distribution of lesions is different. Furthermore, the organism of leprosy is recovered with extreme difficulty, if it can be cultured at all. Rat leprosy of the glandular type presents a lesion which is strikingly similar to that found in our case. On comparing sections of rat leprosy obtained through the courtesy of Dr. R. D. Lillie of the National Institute of Health, we have found that, while there is extensive phagocytosis and macrophagic proliferation, the lymph node structure does not show the widespread alteration that is the rule in our case. The organism of rat leprosy is more difficult to stain and has greater variation in structure than the organism in our case. Moreover, the organism of rat leprosy has not been successfully cultured by ordinary methods.[2]

In Johne's disease there are reacting elements other than the reticuloendothelial cells. The lesions are distributed chiefly throughout the gastro-intestinal tract, and the organism possesses cultural and morphologic characteristics which differentiate it sharply from that responsible for the disease in our case.

A consideration of the mechanism of death in this patient suggests a number of interesting possibilities. The patient obviously was suffering from severe inanition. While it is evident that absorption of nourishment from the gastro-intestinal tract was impaired, it does not seem likely that this factor alone could account for the severe metabolic disturbance. Moreover, the analogy of the situation to that of advanced malignant disease is sharply apparent. Perhaps the same undetermined factors which produce death in certain cases of cancer were operative

in this case. This view finds some support in the extraordinary tumor-like proliferative activity of the reticulo-endothelial cells which constituted a typical feature.

Nature of the Etiologic Agent

Isolation

Isolates of this organism were obtained first from a retroperitoneal lymph node removed for biopsy, later by culture of stools, and finally from the spleen and a lymph node at autopsy. Small acid-fast organisms were seen in smears made from the ileum, jejunum, colon, spleen, abdominal lymph nodes, urine, and ascitic fluid. They did not appear in direct smears from the heart's blood, lungs, liver, kidney, and spinal fluid, although they were subsequently stained in sections of liver and lungs. Post-mortem cultures were freed from contaminants by digestion with normal sodium hydroxide for 30 minutes. Observations were made on isolates obtained both before and after the death of the patient.

Cultivation

Gross Morphologic Features on Solid Media. On glycerin egg medium, growth appeared in 24 to 28 hours as smooth, minute, shiny, pale yellow colonies which rapidly increased in size and pigmentation. After 3 to 7 days the colonies became confluent, definitely elevated, shiny, pale yellow to yellowish orange, and butyrous (Fig. 12). A sweet, yeast-like odor was produced irrespective of the substrate used. Aerial mycelium did not appear at any stage of cultivation, and there was no discoloration of the media. Growth on plated media, while not unlike that on slants, was somewhat slower. On Sabouraud's agar, growth was visible in 36 to 48 hours, and resembled the young colonies produced on glycerin egg slants. As growth increased, however, the color became gray to grayish yellow. With ageing the colonies assumed the characteristic deeper yellow to yellowish orange. The growth eventually became more elevated than on glycerin egg medium, and vermiform (Fig. 13). On plates of sheep's blood-agar, growth was slow, and a slight, irregular hemolysis was produced. Czapek's agar plates or slants with carbohydrate did not support abundant or characteristic growth, although subcultures grew rapidly and well when transferred from this medium to glycerin egg substrate. On Bordet-Gengou agar, colonial development was similar to that obtained on glycerin egg and Sabouraud's media. On Löffler's serum slants, pigmentation of the colonies was increased, so that a deep yellow-orange color was produced. Growth on the Löffler slants was peculiar in that it apparently terminated after

3 to 4 days. Subcultures from this to other media were normal in colony characteristics.

Growth in Liquid Media. Beef extract broth plus 5 per cent glycerin favorably supported growth. In 3 to 7 days a web-like mucoid clot was formed, primarily in the butt of the tube; however, there might be some extension of the growth over the dependent side of the tube. The broth remained clear and there was no pellicle. Prolonged incubation (30 to 40 days) resulted in a slight, increasing turbidity, the degree of which depended upon the amount of inoculum used. The broth was not discolored. In beef extract broth without glycerin and in beef infusion broth without glycerin there was no visible growth after 6 to 7 days. Beef extract broth containing 5 per cent glycerin and 0.5 per cent phenol supported growth which resembled the cultures in glycerinated beef extract broth. Transfers from phenolized broth grew rapidly and well, with no change in gross cultural characteristics. The growth in Sabouraud's broth was similar to that in beef extract broth with glycerin. Proskauer-Beck broth containing asparagin and Dubos' (Tween) broth supported growth, but not as well as did beef broth containing glycerin or Sabouraud's broth.

Viability, and Oxygen and Temperature Requirements. Cultures remained viable for months on slants of glycerin egg medium, and successful transplants were obtained from cultures 1 year old. A culture, with or without other organisms present, resisted digestion by N sodium hydroxide for a period of 30 minutes. The organism was aerobic, but grew in a candle jar containing approximately 10 per cent CO_2. There was no growth in the Brewer anaerobe jar and the organism died within 14 days. The optimum temperature for growth was 37.5°C., but there was good growth at room temperatures. No growth occurred at 40°C., and the organism was killed at 60°C. in 10 minutes.

Biochemical Reactions

In fermentation broth to which 1 per cent xylose or 1 per cent arabinose had been added, acid without gas was produced in 5 to 10 days. No fermentation occurred in broths containing 1 per cent of lactose, dextrose, sucrose, mannite, trehalose, or sorbitol. In litmus milk there was a slight acidity within 20 to 30 days. Peptonization did not occur and growth seemed to be more abundant in the lower portion of the tube. After incubation for 35 days in gelatin, there was neither visible growth nor liquefaction. Indole was not formed, nor was tyrosinase produced, since there was no brownish discoloration of the peptone broth. Nitrates were reduced to nitrites. Cellulose was not decomposed in Dubos' cellulose-nitrate broth, although this medium supported a

slight growth. In potato-dextrose-starch agar little or no growth occurred, and there was no diastasis of the starch.

To determine the utilization of paraffin as the sole source of carbon, a glass rod coated with sterile paraffin was suspended in a flask of carbohydrate-free Czapek's broth. Previous to the insertion of the paraffin-coated rod a 0.2 cc. saline suspension of the organism had been added to the medium. After 7 days' incubation at 37.5°C., the paraffin-covered rod was coated with a dust-like, yellow film. The rod was streaked across the surface of glycerin-egg medium slants, and typical growth of the organism occurred in 4 to 7 days. A stain of a smear from the rod showed many acid-fast organisms.

Microscopic Morphology and Staining Reactions

The organism was pleomorphic, Gram-positive, acid-fast, non-spore-forming, and when undisturbed exhibited true lateral branching. In smears of tissues and exudates from the patient and from experimental animals intracellular bacillary forms predominated, but some free bacillary and a few coccobacillary forms were present. Some of the bacillary forms contained large pleomorphic granules, and all free organisms tended to assume a diphtheroid-like arrangement. There were no ray formations in exudate. In all preparations the organism was 0.2 to 0.45 μ in diameter. The longer elements contained small spherical to ovoid bodies at regular intervals throughout their lengths. These ovoid structures were extremely acid-resistant and Gram-positive. In addition, large pleomorphic structures were found, at times terminally and sometimes irregularly placed throughout the organisms. These were wider than the remainder of the organism. They were not acid-fast, but took the counterstain intensely and were Gram-positive. It may be pointed out that in fat-enriched and glycerinated media the number of these bodies was increased.

On Sabouraud's agar in Van Tieghem cell mounts the organism consistently produced branching filaments in 5 to 8 days. These branches formed a densely matted mycelium in the central portion of the mount. Delicate, tangled, branching hyphae, 0.2 to 0.45 μ in width, were formed at the periphery of the growth (Fig. 16). There was no indication of dichotomous branching, chlamydospores, or chains of spores such as may be seen in those aerobic actinomycetes which produce an aerial mycelium. In cultures other than the Van Tieghem single cell mounts, branching was inconspicuous when examined in smears made by loop transfers (Fig. 15). However, in litmus milk long branching forms were prevalent, and large tangled masses were commonly observed (Figs. 17 and 18). Because of the consistent branching and

the growth on Sabouraud's medium at room temperature, the organism is regarded as a fungus. The small, round to ovoid, regularly placed bodies, therefore, may be interpreted as arthrospores; and the irregularly spaced, pleomorphic, non-acid-fast structures may be termed granules. Therefore, the organism appears structurally as a series of arthrospores bound together by a thin membranous sheath.

Throughout the study of this organism its consistent and extraordinary resistance to decolorization with acid or acid-alcohol was an impressive feature. In smears made from exudate and from freshly isolated cultures the organism was partially decolorized by 25 per cent hydrochloric acid-alcohol* in 1 hour. However, preparations made from litmus milk cultures remained completely acid-fast in 25 per cent hydrochloric acid-alcohol for over 60 minutes. On the other hand, the acid resistance was so diminished by culturing on Sabouraud's medium that the organism was totally decolorized by 2 per cent hydrochloric acid-alcohol in 3 minutes. Acid-fastness was influenced in part by the hydrogen ion concentration of the medium. Increase in either alkalinity or acidity of the medium resulted in a correlated diminution of acid-fastness. The greatest resistance to decolorization was obtained by cultivation at a pH range of 7.2 to 7.6. The age of the culture, however, had no influence, for a 16-months-old culture on glycerin egg medium retained its acid-fastness to a degree comparable to that of fresh cultures.

Regardless of the medium used, the relative acid-fastness of the component parts of the organism was constant. The arthrospores were the most vividly stained by the fuchsin; in contrast the rest of the hypha, although clearly acid-fast, was pale. The granules were never acid-fast.

Analysis of Characteristics

The outstanding features of this organism are its high degree of acid-fastness, its consistent branching on media permitting direct observation, and its resistance to sodium hydroxide digestion. The acid-fastness rivals that found in the mycobacteria and exceeds that usually encountered in the nocardia. However, the acid-fastness of species of Nocardia varies considerably; a few strains have been described as "strongly acid-fast" [3] and "as resistant to acids as the tubercle bacillus." [4] The constant branching of the organism under investigation and its fragility as observed when transferred by loop to smears are suggestive of the aerobic strains of actinomycetes for which Waksman and Henrici [5] employed the generic name Nocardia. It is well known that branching forms of mycobacteria exist. However, they are found

* Prepared by using 25 cc. of C.P. HCl plus 75 cc. of 95 per cent alcohol.

only rarely and under unusual conditions, as in smears from old cultures and sometimes in exudates.[6] This feature of mycobacteria is not consistent even in the same strain. The resistance of our organism to sodium hydroxide digestion is difficult to evaluate as a criterion of classification. This procedure is generally employed to free exudates of organisms other than *Mycobacterium tuberculosis*. However, it may be that the factors responsible for resistance to acid decolorization on the part of an organism may also protect it against sodium hydroxide digestion.

Comparison with Other Organisms

Mycobacterium tuberculosis. As indicated above, the organism here described has a number of morphologic features in common with *M. tuberculosis*. The similarity of fragments of the hyphae to tubercle bacilli is striking, especially when the two are compared in photographs made with the electron microscope. However, the resemblance of our organism to *M. tuberculosis* goes no further than this. The consistency of its branching, its ability to grow on a multitude of media at extremely variable temperatures and hydrogen-ion concentrations, its ability to utilize paraffin as its sole source of carbon, its unique relationship to the cell of its human host, and its failure to kill experimental animals differentiate it sharply from any known form of virulent *M. tuberculosis*.

Other Acid-Fast Bacteria. Intracellular parasitism and acid-fastness, both important characteristics of our organism, are also characteristic of *M. leprae*. Beyond this, however, resemblance between the two completely disappears. The ease with which growth of our organism is obtained would seem to eliminate any possibility of confusing it with *M. leprae* in view of the well known difficulty and special technics required in culturing the leprosy bacillus.[7-9] For example, the organism isolated by Clegg [8] from a case of leprosy is only weakly acid-fast and extremely fastidious in its growth requirements on primary isolation, features which distinguish it sharply from our organism. The organism from Duval's [9] case of leprosy, unlike our organism, required 1 to 7 months on highly enriched media for primary isolation, and the colonies produced were glistening and white. A number of acid-fast bacilli have been isolated from cases of leprosy by other workers, but absolute proof that these organisms are causative of this disease is lacking.[10] From the studies of our own organism in its relation to the disease in the host in which it is found, and in relation to experimentally produced disease in a variety of animals, as described in a subsequent section, there is no question of its etiologic specificity.

Johne's disease of cattle and rat leprosy show intracellular parasitism by acid-fast organisms comparable to that in our case. The organism

of rat leprosy, like that of human leprosy, has not been cultured by ordinary methods,[2] and the organism of Johne's disease differs from our organism in the following ways: Johne's bacillus requires for its growth the addition of extracts of other acid-fast organisms to the primary isolation medium; it requires 4 weeks for growth to become apparent; dull yellowish white, striated colonies are produced; maintenance requires enriched media, and branching has not been observed.[11]

The remaining acid-fast organisms, considered saprophytic mycobacteria, offer problems of comparison with our organism which cannot be solved adequately. For example, statements regarding their ability to branch on Van Tieghem cell mounts are not included in their descriptions. Through the courtesy of Dr. William A. Hagan of Cornell University we have been able to compare our organism with some of these acid-fast bacteria, including one strain each of *M. phlei, M. ranae,* and *M. leprae* (the Clegg II bacillus), and five acid-fast soil saprophytes. For the same purpose, Dr. Hagan also supplied us with three acid-fast organisms isolated from cattle, and one isolated from a case of human leprosy, labeled Kat no. 352. To this collection was added a strain of *M. graminis* obtained through the courtesy of Dr. W. Steenken of the Trudeau Sanatorium. All of these organisms were propagated on Van Tieghem cell mounts, Sabouraud's agar, and glycerin egg medium, and in litmus milk. The organisms were studied both at room temperature and at 37.5°C. Branching, a highly significant characteristic of our organism, was not observed on the cell mounts in the case of *M. graminis, M. phlei, M. ranae,* the Clegg II bacillus, the cattle isolates, and soil saprophyte no. 135; nor was true branching observed in smears made from litmus milk cultures of these organisms. On the other hand, four of the saprophytic soil strains designated by Dr. Hagan as "without identification" [12] and the Kat no. 352 organism showed abundant and true branching. These branching organisms differ from our organism, however, as follows. Kat no. 352 organism and soil saprophyte no. 121 do not grow on Sabouraud's medium. In addition, the Kat no. 352 strain will grow at 47°C., while the organism considered here resists cultivation at 40°C. and beyond. Soil saprophytes nos. 136 and 127 differ from our organism in that they form a pellicle in broth and produce dry, rough colonies on solid media. Soil saprophyte no. 132 differs from our organism in its scant growth on Sabouraud's agar and its dry, flaky colonies. Further studies of these organisms are in progress. However, since they too are unclassified, identification of the organism here reported with one of these, even though unlikely, would be of no assistance in its ultimate classification.

Actinomycetes. The majority of the organisms belonging to the

family Actinomycetaceae may be eliminated from consideration in connection with the identification of our organism because they are not acid-fast. Nevertheless, there is a considerable group of aerobic, slightly acid-fast actinomycetes which grow in the form of branched, vegetative hyphae. These hyphae readily undergo fragmentation and thus give rise to bacillary and coccoid arthrospores. It is to this group of organisms that the generic name Nocardia is given. Since the organism under discussion seems most closely allied to this group, the several species of Nocardia will be discussed in some detail.

In 1888, Nocard [13] described an aerobic, acid-fast, filamentous organism isolated from farcy of cattle which was identified by Gasperini [14] as *Actinomyces farcinicus*. In 1890, Eppinger [15] described *Cladothrix asteroides*, another aerobic acid-fast actinomycete, as the cause of an infection of the brain and meninges in man. This organism was renamed *Nocardia asteroides* and has been designated as the key species of the genus, Nocardia. In 1921, Henrici and Gardner [16] collected reports of 26 instances of human infection by a variety of strains of aerobic acid-fast actinomycetes, Nocardia, and were able to group the organisms into three distinct species. The first of these is *N. asteroides*. This organism is characterized by a mealy growth on agar with pale yellow to deep orange colonies; it does not liquefy gelatin or peptonize milk; it is more highly pathogenic for guinea-pigs than for rabbits. The second species is typified by a strain isolated by Birt and Leishman [17] and later named *N. leishmani* by Chalmers and Christopherson.[18] This organism produces a snow-white growth on solid agar, peptonizes milk, and does not liquefy gelatin. It is pathogenic for guinea-pigs and is reported to be pathogenic for rats and rabbits. The third species is that described by Berestneff.[19] It forms a gray to whitish colonial growth on solid media, liquefies gelatin, and is not pathogenic for laboratory animals. To these three species Henrici and Gardner [16] added a fourth, *A. gypsoides*. Henrici [20] later declared this to be not unlike *N. asteroides*.

In addition to the above-described species, five other species of acid-fast actinomycetes which were recovered from infections in man were found to be described in the literature. From a case of pulmonary actinomycosis, Davis,[21] in 1914, observed such an organism in the sputum of a 64-year-old-man, which he described as an "acid-fast streptothrix." He was not able to culture this organism, nor to determine its pathogenicity for animals. In 1920, Vuillemin [22] isolated an acid-fast, Gram-negative actinomycete from a case of bubonic plague and called it *N. jollyi*. Davis and Garcia,[23] in 1923, isolated an acid-fast organism from subcutaneous abscesses on the extremities of a woman. They assigned their organism to the genus Nocardia, but did not iden-

tify it fully. Later a group of investigators [24] considered this organism akin to *N. asteroides*. In 1934, Gammel,[25] assisted by Werkman, isolated a new species of acid-fast actinomycete from a human infection and, because of its ability to grow in phenolized media, called it *A. phenotolerans*. In 1937 Goldsworthy [26] described an acid-fast actinomycete which was later considered by Kirby and McNaught [27] to be a variant of *N. asteroides*.

The taxonomic distinction between the acid-fast nocardia and the mycobacteria is difficult because of characteristics held in common by organisms of these two groups. This difficulty was acknowledged by Umbreit [28] in his classification, wherein he set up special groups which he called proactinomycetes. He divided this group into the alpha proactinomycetes which are closely related to the mycobacteria and corynebacteria because of their unstable mycelium in slide cultures, and the beta proactinomycetes which are allied to the true actinomycetes because of their stable mycelium and "actinomycete growth in liquid media." The alpha and beta proactinomycetes are further divided into those which are not acid-fast and are therefore akin to the corynebacteria, and those which are acid-fast and are therefore like the mycobacteria. The organism of this present communication can then be classified as one of the acid-fast beta proactinomycetes.

Rosebury [29] classified the actinomycetes according to their parasitic or saprophytic properties, with a species differentiation based on morphologic, environmental, and cultural characteristics. The organism of this report does not lend itself to identification with one of these groups because it possesses both the properties ascribed by Rosebury to the parasitic type and those belonging to the saprophytic types.

The classification of the actinomycetes most useful to us and, we believe, generally acceptable is that of Waksman and Henrici [5] (1943), which is based on the character of the branching of these organisms. In their classification, the family Actinomycetaceae is divided into aerobic and anaerobic genera. The aerobic groups of organisms, partially acid-fast or non-acid-fast, which form a vegetative mycelium dividing by segmentation into bacillary or coccoid elements (arthrospores [30]) but do not produce conidia, are designated Nocardia.

Taxonomic Position of the Etiologic Agent

The placement of the organism dealt with in this report offers difficulty. That it belongs in one of the above-discussed groups of organisms seems obvious because of its acid-fastness. That it is not *M. leprae*, the organism of Johne's disease, or the organism of rat leprosy is indicated

by the ease with which it is cultivated. The degree of its acid-fastness would tend to place it among the mycobacteria; however, the consistency of its branching and its fragmentation into bacillary and coccoid forms would seem to place it among the nocardia. In addition, its ability to grow on Sabouraud's medium at room temperature would appear to confirm the fungous character of this organism. The conclusion is drawn, therefore, that this organism should be more properly considered a species of Nocardia.

In contrasting this organism with the several species of Nocardia, as outlined by Henrici and Gardner,[16] sharp differences become apparent. It differs from *N. asteroides,* the type species of this genus, in that it shows a greater degree of acid-fastness, it resists digestion by sodium hydroxide, it produces buttery colonies, it does not produce aerial mycelia, it does not develop other than yellow pigment, it does not form a pellicle in liquid media, it grows poorly on potato agar and gelatin media, and it fails to produce a lesion in rabbits. It differs from *N. leishmani* in that it does not produce a snow-white growth on agar (that is, it forms no aerial mycelia); it does not produce peptonization of milk, and it is not pathogenic for rabbits. It differs from the species reported by Berestneff [19] because it does not produce gray to white growth on solid media and is pathogenic for laboratory animals. It differs from *A. gypsoides* because it does not produce white colonies, it has no proteolytic activity, it does not produce tyrosinase, and is not lethal to laboratory animals. Equally sharp differences between our organism and those strains which are not included in the classification of Henrici and Gardner are apparent. Since the acid-fast streptothrix described by Davis [21] could not be cultured, and its pathogenicity could not be determined, its differentiation from our organism seems obvious. *N. jollyi,* reported by Vuillemin,[22] proved to be Gram-negative, which differentiates it from the organism here reported. *A. phenotolerans,* of Gammel and Werkman,[25] is similar to our organism in that it was extremely acid-fast and capable of growing in a medium to which was added 0.5 per cent phenol, but it differs in that it formed a pellicle in liquid media and produced aerial mycelia.

Summary

Inasmuch as this organism is considered a species of Nocardia, and inasmuch as there are sharp differences between it and known species of this genus, it is concluded that this organism represents a hitherto undescribed species; for this new species the name *Nocardia intracellularis, n. sp.,* is suggested because of the characteristic intracellular

position which it occupies in the human host. A brief characterization in Latin with English translation is as follows:

Nocardia intracellularis, sp. nov. Filamentis ramosis, in fragmenta secendentibus, ex baculis seriatis constitutis, 0.2-0.45 μ latis; in culturis in liquoribus colonias ramosas efformat; apice non clavatis; chlamydosporis non efformantibus; arthrosporis ellipsoideis apice efformantibus; non-motilibus. Hyphae colorantur per fuchsinam acidospiritu-rectificato haud decolorantur. Difficulter propagatur in gelatina, atque gelatinam non liquefacit. In culturis agar-agar applantis colonias circulares, elevatas, nitentes, leves, non mucosas, post 3-6 dies.

Fluidium hyalinium in culturis liquidis glycerino atque massas, albas, mucosas, ad basem tubuli efformantis. Lac coloratum cum litmo, acidum fit post 20-30 dies. In culturis Sabouraudii vegetat rapide post 3-7 dies. Amylum non vertit. Decompositionem cellulosae non provocat. Tyrosinase non efficit. Indole non efficit. Decompositionem potassae nitrasi non provocat. Non viget in infusionibus tuberorum solani agar-agar. Crescit in jure carnis in quo glycerino atque 0.5 per centum acidum carbolicum adest. In oxygenii absentia non evolvitur sed in 10 per centum carbonei dioxidi viget. Cera origine sola carbonis utor potest. Viget rapide ad temperaturam 37.5°C. Facile evolvitur usque ad temperaturam 40°C.

Habitat: In granulomis organorum infectorum lymphaticorum observatus est, ac in faecibus homini vivi in quo morbum causart, atque in cadavero in laesionibus granulomatis disseminatis late quas edit.

Nocardia intracellularis, n. sp. Filaments branched, becoming fragmented, composed of bacillary elements in series, 0.2 to 0.45 μ in width; in cultures in liquids, branched colonies are formed; not club-shaped at the tips; and lacking chlamydospores; nonmotile. The hyphae are not at all discolored when stained with fuchsin and treated with acid alcohol. It grows poorly in gelatin, which it does not liquefy. In agar plate cultures the colonies are circular, raised, wet-shining, smooth, and non-mucoid after 3 to 6 days.

In glycerin broth the fluid remains clear; and white, mucoid masses are formed at the bottom of the tube. Litmus milk becomes acid after 20 to 30 days. On Sabouraud's agar it grows well after 3 to 7 days. Starch is not changed. It does not decompose cellulose. Tyrosinase is absent, and indole is not produced. It does not decompose nitrates of potassium. It does not thrive on potato agar. It grows in beef extract broth to which has been added glycerin and 0.5 per cent carbolic acid. It does not develop in the absence of oxygen, but grows in an atmosphere having 10 per cent CO_2. It can use paraffin as a sole source of carbon. It thrives at 37.5°C. and tolerates well temperatures up to 40°C.

Habitat: Observed in granuloma of infected lymph nodes and in the feces of a living patient whose death it caused. Observed also at autopsy in widely disseminated granulomatous lesions which it produced.

PATHOGENICITY OF NOCARDIA INTRACELLULARIS

The study of the reaction of animals to inoculation by *Nocardia intracellularis* was divided into three parts: The determination of species susceptibility; a study of the route of infection; and a study of the development of the lesion.

Species Susceptibility

In the experiments designed to determine which animals would become infected, chickens, rabbits, goldfish, frogs, rats, mice, and guinea-pigs were used (Table I). The standard inoculum was 0.2 cc. of a suspension of viable organisms in a concentration of 30 organisms per oil-immersion field.

Six chickens were inoculated, 3 by intravenous injection and 3 by intrathoracic injection. They were sacrificed after 10 weeks. Lesions were not found in any of these animals.

Six rabbits were inoculated, 2 by intravenous, 2 by intraperitoneal, and 2 by subcutaneous injection. One rabbit, intravenously inoculated, died 3 weeks later of septicemia, staphylococci being recovered from the blood stream. The site of inoculation showed no reaction. The remaining 5 rabbits were skin-tested and sacrificed after 10 weeks. The average weight gain was 655 gm. Lesions were not found in any of these animals.

Twelve goldfish were used. In 6 the inoculum was injected intraperitoneally, and in 4 it was injected into the dorsal lymph sac. Lesions failed to form in all cases.

Six frogs, *Rana cetesbiana,* were used. In 3 the inoculum was injected intraperitoneally, and in 3 it was injected into the dorsal lymph sac. Lesions failed to form in all cases.

Ten rats were used: 5 were injected subcutaneously and 5 intraperitoneally. Among the group injected subcutaneously there was one which died 1 week later. Its body was not recovered. The remaining 4 were sacrificed 10 weeks later after a negative skin test. These animals showed an average weight gain of 120.9 gm. In one there was proliferation of lymphoid tissue in the spleen, in which acid-fast organisms could be stained. Two other rats showed focal accumulations of epithelioid cells in the lymph nodes. In these foci acid-fast organisms were stained, some of them showing "Y" forms (Fig. 19). The last rat had no lesions. Among the group inoculated by intraperitoneal injection, one rat died of pneumonia in 8 days. Proliferation of macrophages with giant cell formation occurred at the site of inoculation. Acid-fast organisms were stained, some of which were "Y" forms. The remain-

TABLE I

Species-Susceptibility to the Organism

Animal	Route of infection	Weight		Sensitivity	Death	Significant lesions	Organisms stained in tissues
		Initial	Death				
		gm.	*gm.*				
Mouse 1	Subcutaneous	15.6	21.3	Negative	Sacr. 72 days	None	No
Mouse 2	Subcutaneous	14.8	21.0	Not done	Died 33 days	Pneumonia, granuloma of lymph nodes	Yes
Mouse 3	Subcutaneous	14.4	16.7	Negative	Died 40 days	Pneumonia, granuloma of liver	Yes
Mouse 4	Subcutaneous	14.3	16.5	Negative	Sacr. 72 days	None	No
Mouse 5	Subcutaneous	14.8	17.9	Negative	Died 45 days	Pneumonia, focal necrosis of liver	No
Mouse 6	Intraperitoneal	11.4	9.7	Not done	Died 26 days	Granuloma of intestines, liver, lymph node	Yes
Mouse 7	Intraperitoneal	8.4	7.5	Not done	Died 6 days	Focal necrosis of liver, hemorrhage in abdomen	No
Mouse 8	Intraperitoneal	9.4	9.4	Not done	Died 1 day	Peritoneal hemorrhage	No
Mouse 9	Intraperitoneal	13.2	13.2	Not done	Died 2 days	Granuloma, mesenteric lymph nodes	Yes
Mouse 10	Intraperitoneal	12.3	11.6	Negative	Died 34 days	None, focal necrosis of liver	No
Rat 1	Subcutaneous	27.8	26.6	Not done	Died 7 days(?)	No remains	No
Rat 2	Subcutaneous	48.0	191.6	Negative	Sacr. 69 days	Granuloma in spleen	Yes
Rat 3	Subcutaneous	35.0	134.2	Doubtful	Sacr. 69 days	Granuloma of lymph node	Yes
Rat 4	Subcutaneous	67.2	192.2	Doubtful	Sacr. 69 days	None	No
Rat 5	Subcutaneous	44.3	160.2	Negative	Sacr. 69 days	Granuloma of lymph node	Yes
Rat 6	Intraperitoneal	67.2	192.2	Doubtful	Sacr. 69 days	Granuloma of lymph node	Yes
Rat 7	Intraperitoneal	29.8	182.7	Negative	Sacr. 69 days	None	No
Rat 8	Intraperitoneal	46.5	74.8	Not done	Died 8 days	Granuloma at site of inoculation	Yes
Rat 9	Intraperitoneal	44.9	161.5	Positive	Sacr. 69 days	Granuloma of lymph nodes	Yes
Rat 10	Intraperitoneal	40.3	150	Doubtful	Sacr. 69 days	None	No
Guinea-pig 1*	Subcutaneous	575	690	Positive	Sacr. 70 days	Granuloma at site of inoculation	Yes
Guinea-pig 2*	Subcutaneous	380	525	Positive	Sacr. 70 days	Granuloma, lymph nodes and liver	Yes
Guinea-pig 3*	Subcutaneous	350	450	Positive	Sacr. 70 days	Granuloma, lymph nodes, epididymis, and liver	Yes
Guinea-pig 4*	Subcutaneous	420	575	Positive	Sacr. 70 days	Granuloma, liver and gastrointestinal tract	Yes

Guinea-pig 5*	Subcutaneous	535	650	Positive	Sacr. 70 days	Granuloma, epididymis and lymph nodes	Yes
Guinea-pig 6*	Subcutaneous	515	600	Positive	Sacr. 70 days	Granuloma, lymph nodes, liver, and brain	Yes
Rabbit 1908	Intravenous	2175	1575	Not done	Died 21 days	Pneumonia, focal necrosis of liver	No
Rabbit 1911	Intravenous	2325	3150	Negative	Sacr. 68 days	None	No
Rabbit 1913	Intraperitoneal	2025	2900	Negative	Sacr. 68 days	None	No
Rabbit 1919	Intraperitoneal	2275	3100	Negative	Sacr. 68 days	None	No
Rabbit 1922	Subcutaneous	1925	2250	Negative	Sacr. 68 days	None	No
Rabbit 1923	Subcutaneous	2375	2900	Negative	Sacr. 68 days	None	No
Chicken 1	Intravenous				Sacr. 69 days	None	No
Chicken 2	Intravenous				Sacr. 69 days	None	No
Chicken 3	Intravenous				Sacr. 69 days	None	No
Chicken 4	Intrapleural				Sacr. 69 days	None	No
Chicken 5	Intrapleural				Sacr. 69 days	None	No
Chicken 6	Intrapleural				Sacr. 69 days	None	No
Frog 1	Dorsal lymph sac				Died 6 days	None	No
Frog 2	Dorsal lymph sac				Sacr. 60 days	None	No
Frog 3	Dorsal lymph sac				Sacr. 60 days	None	No
Frog 4	Intraperitoneal				Died 20 days	None	No
Frog 5	Intraperitoneal				Sacr. 60 days	None	No
Frog 6	Intraperitoneal				Died 28 days	None	No
Goldfish 1	Intraperitoneal				Sacr. 92 days	None	No
Goldfish 2	Intraperitoneal				Sacr. 92 days	None	No
Goldfish 3	Intraperitoneal				Sacr. 92 days	None	No
Goldfish 4	Intraperitoneal				Sacr. 92 days	None	No
Goldfish 5	Intraperitoneal				Sacr. 92 days	None	No
Goldfish 6	Intraperitoneal				Sacr. 92 days	None	No
Goldfish 7	Tail				Sacr. 92 days	None	No
Goldfish 8	Tail				Sacr. 92 days	None	No
Goldfish 9	Tail				Sacr. 92 days	None	No
Goldfish 10	Tail				Sacr. 92 days	None	No
Goldfish 11	Organisms in water				Sacr. 92 days	None	No
Goldfish 12	Organisms in water				Sacr. 92 days	None	No

* A moderate swelling appeared at site of inoculation, which receded during the third week.

ing 4 rats were sacrificed after 10 weeks. The average weight gain was 131.9 gm. Two of these animals showed focal accumulations of epithelioid cells in the lymph nodes, from which acid-fast organisms were re-isolated. The other 2 showed no lesions.

Ten mice were used; 5 were injected subcutaneously, and 5 intraperitoneally. In the first group one died at 5 weeks. This animal showed small collections of epithelioid cells in the lymph nodes. Death was due to pneumonia. A second mouse died of pneumonia, also. There were focal collections of macrophages in the lymph nodes with focal necrosis in the liver, but acid-fast organisms could not be demonstrated. A third mouse died after 7 weeks. No lesions were found which could be ascribed to infection by acid-fast organisms. Two mice lived 10 weeks, whereupon they were sacrificed but no lesions were found. The average weight gain was 3.95 gm. Among the group intraperitoneally injected, 2 died by the second day of hemorrhage. In one, acid-fast organisms formed a focal aggregate in a mesenteric lymph node. No other lesions were found. A third mouse died at 6 days of peritoneal hemorrhage. A fourth died at 4 weeks. In this mouse there was macrophagic proliferation in the mesenteric nodes and those surrounding the intestine, with small tubercle-like lesions containing acid-fast organisms in the liver. A fifth mouse died at 5 weeks. No lesions referable to acid-fast organisms were found, but there was focal necrosis in the liver.

Six guinea-pigs were injected subcutaneously. In each a large swelling characterized by an epithelioid reaction of large macrophages and by central necrosis appeared at the site of injection. Acid-fast organisms invariably were isolated in cultures and were stained in histologic sections. Lesions in the guinea-pig differed from those in the patient in that, while the acid-fast organisms usually were arranged in clumps, massive intracellular parasitism was not found. The inguinal lymph nodes in 3 and the tracheobronchial lymph nodes in 4 of the 6 guinea-pigs showed a granulomatous reaction in the form of marked hyperplasia of the macrophages. In these nodes acid-fast organisms were stained. In 4 animals a reaction of the same kind was found in the liver (Fig. 20). These granulomas consisted of an aggregation of macrophages with a central area of necrosis. Inflammatory cells of other types were rare. Three animals showed diffusely scattered areas of focal necrosis of the liver. The lymphoid elements of the gastrointestinal tract showed uniformly a proliferative reaction in which acid-fast organisms were stained in only 2 instances. The lungs also uniformly showed proliferation of the lymphoid elements surrounding the vessels and the bronchi (Fig. 21), but in only one lung were acid-

fast organisms stained. In one guinea-pig there was a characteristic lesion in the wall of the gallbladder, and also lesions appeared in the epididymis (Fig. 22). In one guinea-pig a small group of epithelioid cells was found in the meninges of the basal portion of the brain. In this tubercle-like lesion acid-fast organisms were stained. There was one instance of epithelioid reaction in the spleen in which acid-fast organisms were found; however, in all animals there was a diffuse proliferation of lymphoid elements. Lesions did not appear in the heart, kidney, or adrenal of any inoculated guinea-pig.

Route of Infection

For the purpose of investigating the route of infection, guinea-pigs were selected. Groups of 4 animals were inoculated in each of the following ways: By intravenous, intraperitoneal, and subcutaneous injection; by ingestion; and by instillation into the conjunctival sac (Table II).

In the group inoculated intravenously, the lungs showed proliferation of lymphoid elements in 3 animals, with the formation in one of a tubercle-like lesion carrying acid-fast organisms. One guinea-pig showed no lesion in the lung. Lymph nodes in 2 showed hyperplasia of the reticulo-endothelial elements, but carried no acid-fast organisms. In the livers there were 2 instances of marked reaction in the form of proliferation of macrophages with a central area of necrosis, one of phagocytosis of acid-fast organisms by the Kupffer cells, and one in which there was no lesion. The spleens of these animals showed uniform proliferation of the lymphoid elements, and in one instance acid-fast organisms were seen intracellularly. One of these guinea-pigs showed a granulomatous lesion in the epididymis typical of the kind produced by this organism.

In the group receiving intraperitoneal injections there was uniform hyperplasia of the lymphoid elements in the lungs, lymph nodes, and spleens. In the liver of one of these animals there were groups of epithelioid cells containing scattered acid-fast organisms. In 2 animals the same lesion appeared in the lymph nodes, but the fourth animal showed no lesions, and no acid-fast organisms were stained.

In the group inoculated by subcutaneous injection there was produced uniformly an area of necrosis surrounded by large mononuclear macrophages at the site of inoculation. With the exception of regional inguinal nodes, the lymph nodes were hyperplastic in only one instance. In the livers of 2 animals were found lesions, consisting of large mononuclear phagocytes, with a central area of necrosis. Acid-fast organisms

TABLE II

Route of Infection

Guinea-pig no.	Route of infection	Weight		Sensitivity	Sacrificed after	Significant lesions	Organisms stained
		Initial	Death				
		gm.	gm.				
1876	Intravenous	700	625	++	84 days	Granuloma of epididymis	Yes
1890	Intravenous	475	450	++	84 days	Granuloma of lungs and liver	Yes
1882	Intravenous	725	700	++	84 days	Phagocytosis by Kupffer cells and macrophages of spleen	Yes
1895	Intravenous	500	450	++	84 days	Granuloma of liver	Yes
1879	Intraperitoneal	400	600	++	81 days	None	No
1892	Intraperitoneal	400	550	++	81 days	Granuloma of lymph node	Yes
1900	Intraperitoneal	400	675	++	81 days	Phagocytosis of acid-fast organisms in lymph nodes	Yes
1913	Intraperitoneal	325	700	++	81 days	Tuberculoid reaction in liver	Yes
1918*	Subcutaneous	650	675	++	84 days	Granuloma of liver	Yes
1924*	Subcutaneous	675	700	++	84 days	None	Yes
1901*	Subcutaneous	725	925	++	84 days	Healing lesion at site of inoculation	No
1902*	Subcutaneous	300	400	++	84 days	Granuloma of liver	Yes
1906	Ingestion	625	725	o	82 days	Granuloma of liver, lungs, intestines	Yes
1921	Ingestion	350	525	++	82 days	Acid-fast organisms stained in liver	Yes
1905	Ingestion	400	550	++	82 days	Granuloma of lungs and lymph nodes	Yes
1903	Ingestion	425	500	++	82 days	Acid-fast organisms stained in lungs, liver, and lymph nodes	Yes
1907	Instillation, eye	500	650	?	85 days	None	No
1909	Instillation, eye	775	725	++	85 days	Granuloma of lungs, acid-fast organisms stained in spleen	Yes
1915	Instillation, eye	375	375	?	85 days	None	No
1920	Instillation, eye	350	460	++	85 days	Granuloma of lymph node and spleen	Yes

* Showed granulomatous inflammation at site of inoculation. The lesion was healed at autopsy.

were stained in the necrotic areas. The spleen was uniformly hyperplastic, but no acid-fast organisms were found. None of these animals showed lesions in the epididymis.

In all 4 of the animals which had ingested the inoculum the quantity of lymphoid tissue in the lungs was striking. Three showed lymph nodes with extensive proliferation of macrophages, and in 2 areas of proliferation many acid-fast organisms were stained. These organisms did not occupy an intracellular position. The livers of 2 guinea-pigs showed acid-fast organisms within the Kupffer cells, but no granulomatous lesions. Two animals showed extensive proliferation of the lymphoid tissue of the spleen, and in one spleen acid-fast organisms were stained. The intestines of 2 guinea-pigs showed marked proliferation of the lymphoid follicles, and, in one, acid-fast organisms were demonstrated in the follicles. The kidneys of 2 animals showed nodules of epithelioid cells in which acid-fast organisms were found.

Only 2 of the 4 animals which had received instillation of inoculum into the conjunctival sac showed infection. In one there was conspicuous lymphoid proliferation in the lymph nodes and spleen, the latter containing acid-fast organisms. The other, in addition, showed a granulomatous reaction in the lung. This lesion consisted of a marked proliferation of the large macrophages with acid-fast organisms scattered throughout.

All of the animals used in this experiment were inoculated intradermally with a suspension of organisms killed by autoclaving. This inoculation produced a reaction about 24 hours after the injection. The reaction consisted of swelling and induration with a broad area of erythema. In the 2 guinea-pigs which resisted infection by ingestion of the inoculum there was no reaction. Histologically, the sites of reaction consisted of a central zone of necrosis and liquefaction, with polymorphonuclear leukocytes. In the focus of polymorphonuclear leukocytes numerous fragmented, acid-fast granules were demonstrated. Surrounding this zone of polymorphonuclear response there was extensive proliferation of macrophages, some of which contained large numbers of acid-fast granules. There was, in addition, an infiltration by lymphocytes and eosinophils with fibroblastic proliferation.

Development of the Lesion and Course of the Infection

For the purpose of studying the development of the lesion and course of the infection, 18 mice and 24 guinea-pigs were used. These animals were inoculated subcutaneously with 0.2 cc. of a suspension containing approximately 30 living organisms per oil-immersion field. They were sacrificed, 2 at a time, at weekly intervals (Table III).

TABLE III

Course of the Infection *

Animal	Sensitivity	Death	Significant lesions	Organisms stained in tissues
Guinea-pig A1	Negative	Sacr. 9 days	Healing scar at site of inoculation	No
Guinea-pig A2	Negative	Sacr. 9 days	Healing scar at site of inoculation	No
Guinea-pig A3	Not done	Died 11 days	Pneumonia; healing at site of inoculation	No
Guinea-pig A4	Not done	Died 11 days	Healing at site of inoculation	No
Guinea-pig A5	Not done	Died 11 days	Healing at site of inoculation	No
Guinea-pig A6	Not done	Died 12 days	Pneumonia; healing at site of inoculation	No
Guinea-pig A7	Negative	Sacr. 16 days	Nothing	No
Guinea-pig A8	Negative	Sacr. 16 days	Nothing	No
Guinea-pig A9	Not done	Died 18 days	Pneumonia; healing at site of inoculation	Yes
Guinea-pig A10	Not done	Died 18 days	Pneumonia; healing at site of inoculation	Yes
Guinea-pig A11	Not done	Died 18 days	Pneumonia; healing at site of inoculation	No
Guinea-pig A12	Not done	Died 18 days	Nothing	No
Guinea-pig A13	Not done	Died 21 days	Nothing	No
Guinea-pig A14	Not done	Died 23 days	Pneumonia	No
Guinea-pig A15	Negative	Sacr. 23 days	Nothing	No
Guinea-pig A16	Negative	Sacr. 23 days	Nothing	No
Guinea-pig A17	Negative	Sacr. 30 days	Granuloma of lymph nodes	Yes
Guinea-pig A18	Negative	Sacr. 30 days	Focal proliferations in lymph nodes	No
Guinea-pig A19	Not done	Died 32 days	Pneumonia	No

Guinea-pig A20	Doubtful	Sacr. 37 days	Focal proliferation in lymph nodes	Yes
Guinea-pig A21	++	Sacr. 37 days	Granuloma of lymph nodes and liver	Yes
Guinea-pig A22	++	Sacr. 42 days	Granuloma of lymph nodes and necrosis of liver	Yes
Guinea-pig A23	++	Sacr. 42 days	Peculiar macrophagic response in lungs	No
Guinea-pig A24	++	Sacr. 42 days	Nothing	No
Mouse A1	Not done	Died 1 day	Necrosis of liver	No
Mouse A2	Not done	Died 7 days	Granuloma of skin at site of inoculation	Yes
Mouse A3	Negative	Sacr. 9 days	Granuloma of skin at site of inoculation	Yes
Mouse A4	Negative	Sacr. 9 days	Granuloma of skin at site of inoculation	Yes
Mouse A5	Not done	Died 11 days	Healing skin lesion at site of inoculation	No
Mouse A6	Negative	Sacr. 16 days	Healing skin lesion at site of inoculation and lymph node	Yes
Mouse A7	++	Sacr. 16 days	Healing skin lesion at site of inoculation	No
Mouse A8	+++	Sacr. 23 days	Healing skin lesion at site of inoculation and lymph nodes	Yes
Mouse A9	+	Sacr. 23 days	Healing skin lesion at site of inoculation	Yes
Mouse A10	+	Sacr. 30 days	Nothing	No
Mouse A11	+	Sacr. 30 days	Nothing	No
Mouse A12	Not done	Died 33 days	Nothing	No
Mouse A13	++	Sacr. 37 days	Nothing	No
Mouse A14	++	Sacr. 37 days	Nothing	No
Mouse A15	o	Sacr. 44 days	Granuloma of lymph node	Yes
Mouse A16	+	Sacr. 44 days	Scars in spleen and lymph node	No
Mouse A17	o	Sacr. 51 days	Nothing	No
Mouse A18	++	Sacr. 51 days	Nothing	No

* All animals developed a swelling at the site of inoculation. This swelling gradually subsided after about 2 to 3 weeks.

The mice uniformly showed a slight swelling and occasionally ulceration at the site of inoculation (Fig. 23). Biopsy of one of these lesions showed the characteristic reaction, described in the preceding section. In the areas of necrosis were many acid-fast organisms. Mice which were sacrificed on the 11th day showed disappearance of the central area of necrosis and replacement by a granulomatous reaction of a different form, consisting of large mononuclear phagocytes with Langhans' giant cells. Those sacrificed on the 23rd day showed a granulomatous reaction in the liver and spleen. In those sacrificed on the 30th day, the site of inoculation was completely healed, and in its place only scar tissue was found. Internally these mice showed only extensive hyperplasia of the lymphoid elements of the liver and lymph nodes. Acid-fast organisms were not found. In none of the mice sacrificed subsequently were active lesions found, with the exception of one sacrificed on the 44th day. In this mouse there was an extensive macrophagic proliferation of the regional lymph nodes. Acid-fast organisms were found in an intracellular position in these nodes. In one mouse a large area of scar tissue was found in the spleen. This was thought to be a healed lesion. Three of these mice showed healed, tubercle-like lesions in the liver.

The guinea-pigs, like the mice, exhibited a granulomatous reaction at the site of inoculation. Those sacrificed between the 9th and the 18th day showed healing at the site of inoculation. Guinea-pigs sacrificed on the 30th and 37th days showed internal granulomatous reactions; 2 had focal epithelioid proliferation in the lymph nodes. One showed a lesion of this type in the liver, and proliferation of the reticulo-endothelial elements of the lung, which contained acid-fast organisms. In all 4 there was hyperplasia of the lymphoid elements of the spleen. One animal, sacrificed on the 42nd day, showed a granulomatous reaction in a lymph node in which acid-fast organisms were stained. Acid-fast organisms also were stained in the lung, and there were extensive areas of necrosis in the liver. All guinea-pigs sacrificed subsequent to the 37th day were without active lesions.

The course of infection in the experimental animal was different from that in the patient. At the site of inoculation there developed an area of necrosis surrounded by large mononuclear macrophages. This lesion healed within about 3 weeks. Throughout the phase of healing the lesion was characteristically granulomatous, with proliferation of macrophages and formation of giant cells. The organism occasionally would be found in a "Y" form in such lesions. The infec-

tion usually remained localized. However, in many animals small isolated lesions were produced in lungs, liver, spleen, and intestine. In the course of 4 to 5 weeks all lesions healed, and sensitivity of the tuberculin type was produced. The infection did not kill the animal.

The guinea-pigs used in this experiment were tested by intradermal inoculation of organisms killed by heat. No sensitivity developed until the 30th day of the infection. These animals had a slight reaction, and all sacrificed subsequently showed a reaction of the tuberculin type, which became distinct by the 42nd day.

Summary of the Studies on the Pathogenicity of Nocardia intracellularis, n. sp.

Nocardia intracellularis, n. sp., was found to be pathogenic * for guinea-pigs, rats, and mice, but it produced no lesion in rabbits, chickens, frogs, or goldfish. Infection of guinea-pigs by this organism was successful when it was injected subcutaneously, intravenously, and intraperitoneally, and when fed by mouth; but when it was instilled into the conjunctival sac infection was successful in only 50 per cent of animals. The animals for which *N. intracellularis, n. sp.*, is pathogenic uniformly showed a local reaction at the site of inoculation consisting of proliferation of macrophages with necrosis in the center of the lesion but with little intracellular parasitism. Ten per cent of these animals had a systemic distribution of the lesions, chiefly in the liver, lungs, and lymph nodes. These lesions healed after about 6 weeks, at which time the animals exhibited a tuberculin-like skin sensitivity to a heat-killed suspension of the organism.

General Summary and Discussion

In summary, the condition described is a new granulomatous disease entity caused by an unusual acid-fast organism. The source of the infection is not known. Inasmuch as the most numerous and the largest lesions were found in the retroperitoneal and mesenteric lymph nodes and in the lymphoid tissue of the intestines, the gastro-intestinal tract is suggested as the portal of entry. The characteristic reaction to the organism consisted of phagocytosis of the pathogen by large mononuclear phagocytes with proliferation of these cells and the production of multinucleated giant cells. The only other basic pathologic process represented was necrosis of a coagulative type. In the absence of poly-

* For the purpose of this report, pathogenic is defined as "capable of producing a disturbance in function or structure of any organ or part of the body" and does not imply that the disease must be lethal.

morphonuclear leukocytes and even lymphocytic infiltration, this lesion may be regarded as a pure form of granuloma.[31] This granuloma exhibited no consistent morphologic structure such as tubercle formation, but rather presented itself simply as a diffuse proliferation of macrophages.

In spite of its ability to resist the digestive activity of macrophages and to multiply to an extraordinary degree within these cells, the organism appeared to elaborate little that was toxic to its host. The damage done by it seemed to arise solely out of its ability to grow and multiply within reticulo-endothelial cells of the host, which relationship gave rise to an extraordinary and almost unlimited proliferation of these cells. The initial reaction to this organism was phagocytosis, followed by proliferation of the phagocytes. The end-result of this ultimate form of parasitism is death of the host cells.

The major systemic effects seemed to depend upon the specific location of the larger and more numerous lesions. Their position in the lymphatic system of the gastro-intestinal tract produced obstruction of the lymphatics and consequently failure of absorption of fat. This failure was reflected in the engorged state of the lymphatics and had produced, in part, the systemic effect of inanition. In addition to the nutritional disturbance, there may be other factors in the production of inanition. The extreme degree of proliferation of the macrophages and the clinical course suggested a neoplasm. Indeed, had the presence of organisms not been demonstrated, one would have been justified, from the history and gross examination of the lesion, in considering this disease a peculiar form of lymphosarcoma. In view of this superficial similarity to a neoplasm, the other unknown factors in the production of death might be similar to those with cancer.

Placement of this organism within an established classification has been exceedingly difficult. The degree of acid-fastness and its ability to withstand sodium hydroxide digestion have suggested that it is related to the mycobacteria. However, that it branches consistently and early, that it grows on Sabouraud's medium at room temperature, that it utilizes paraffin as a sole source of carbon, and that it grows rapidly and well on a multiplicity of media seem to identify it more closely with the actinomycetes. Our difficulty in choosing between these two groups is in harmony with the generally prevailing uncertainty of the position of acid-fast actinomycetes. Many have considered them to be mycobacteria, and many have considered mycobacteria to be fungi.[32-35] Into this controversy we prefer not to enter, but the organism seems

most closely related to the genus designated Nocardia, which for the time being is assigned to the family Actinomycetaceae. Since we have been unable to find a reported species with which it is identical, we have concluded that this organism represents an undescribed species of Nocardia. Because its presence within the cells whose reactivity it provokes in its human host is so constant, so typical, and so impressive, we have utilized this characteristic in assigning the name *Nocardia intracellularis, n. sp.*, to it.

Lesions have been produced by this organism in guinea-pigs, rats, and mice, but not in rabbits, chickens, frogs, or goldfish. The inoculation lesion consists of a central zone of necrosis in which groups of organisms may be found. This zone is surrounded by macrophages, in which there are very few organisms. On the outer edge of this macrophagic zone is fibroblastic proliferation. In only about 10 per cent of infections is there systemic spread. When found, the systemic lesions are histologically similar to the local lesion. There have been no fatal infections in these animals. The chief difference between the reaction in the experimental animal and in man is the relative paucity of intracellular organisms and the relative abundance of necrosis in the experimental animal.

The organism under consideration has been contrasted with 37 other acid-fast actinomycetes with respect to the lesion which they produce and the animals for which they are pathogenic (Table IV). Thirty of these organisms were isolated from human sources. Nineteen of these were obtained from abscesses, of which 13 occurred in the lungs. Draining sinuses, including those of madura foot, were the source of three of these organisms. One was obtained from a case of postoperative peritonitis. Three were found in pulmonary lesions which were called bronchopneumonia, and one was isolated from "cirrhotic nodules of the lungs." There was one from a case "resembling plague," one from a case of "fibrosis of the spleen," and one from a patient who did not die, but whose x-ray findings suggested tuberculosis.

From this summary of cases it is seen that acid-fast actinomycetes have been isolated from a wide variety of human lesions. The only case said to be granulomatous was that of Gammel,[25] but the lesions which he described were not similar to those of the patient here presented. In fact, we have been unable to find in the literature a description of any case which is essentially similar.

All of the 37 organisms referred to above show a striking variability in pathogenicity for animals. The variation ranged from lethal patho-

TABLE IV

Source and Animal Pathogenicity of Nocardia intracellularis, n. sp., Contrasted with 37 Reported Acid-Fast Actinomycetes

Author	Name of organism	Source	Pathogenic for	Nonpathogenic for
Eppinger[15]	*A. asteroides*	Abscess of lungs and brain	Guinea-pig, rabbit	Mouse
Aoyama and Miyamoto[36]	*A. asteroides*	Abscess of lungs	Guinea-pig	Fowl, rabbit, mouse
Buchholz[37]	*A. asteroides*	Abscess of lungs	Not stated	
Foulerton[34]	*A. asteroides*	Abscess of lungs		Guinea-pig
Horst[38]	*A. asteroides*	Abscess of lungs	Guinea-pig, rabbit	Mouse
Loehlein[39]	*A. asteroides*	Abscess of heart, lungs, brain	Guinea-pig, rabbit	Dog
MacCallum[40]	*A. asteroides*	Postoperative peritonitis	Mouse, dog, guinea-pig, rabbit	
Schabad[41]	*A. asteroides*	Abscess of lungs and chest wall	Guinea-pig, rabbit	Pigeon, mouse
Stokes[4]	*A. asteroides*	Abscess of lungs	Guinea-pig, rabbit	
Birt and Leishman[17]	*A. asteroides**	"Cirrhotic nodules" of lung	Guinea-pig	
Musgrave and Clegg [42]	*A. asteroides*†	Madura foot	Dog, monkey, guinea-pig	Rabbit
Sabrazès and Rivière[43]	*A. asteroides*(?)	Abscess of brain	Guinea-pig(?), rabbit(?)	Rat
Sabrazès and Rivière[43]	*A. asteroides*(?)	Pleuropneumonia		
Berestneff[19]	*A. rivierei* (Brumpt)	Abscess of brain	Guinea-pig(?), mouse(?), dog(?), rabbit(?)	
Vuillemin[32]	*A. jollyi*	Case resembling plague	Not stated	
Butterfield[44]	*A. anaerobies*	Abscess of lungs	Not stated	
De Kortê[45]	*A. cylindraceus*	Draining sinus of ear	Not stated	
Flexner[46]	*A. pseudotuberculosis*	Abscess of lungs	Guinea-pig(?)	
Gammel[25]	*A. phenotolerans*	Acne of face	Guinea-pig	
Gibson[47]	*A. gibsoni*	Fibrosis of spleen	Not stated	
Greco[48]	*A. sommeri*	Mycetoma pedis	Rabbit, guinea-pig	
Henrici and Gardner[16]	*A. gypsoides*	Bronchopneumonia	Guinea-pig	
Ferré and Faguet[49]	*A. sabrazes*(?)	Cerebral abscess	None	Rabbit, guinea-pig
Bernstein[50]	Unnamed	Abscess of lungs	Guinea-pig, rabbit	
Davis[21]	Unnamed	Sputum in case with radiograph similar to tuberculosis	Rabbit, mouse	
Davis and Garcia[23]	Unnamed	Subcutaneous abscess	Rabbit, guinea-pig, rat, mouse	
Goldsworthy[26]	Unnamed	Lung abscess		
Ljubimoff[51]	Unnamed	Bronchopneumonia	Not stated	
Scheele and Petruschky[52]	Unnamed	Abscesses of lung and skin	Guinea-pig(?), rabbit(?)	
Ribytkof and Maloletkof[53]	Unnamed	Abscess of spinal cord		
De Mello and St. Antonio Fernandes[54]	*A. chalmersi*	Saliva of horse	Not stated	

Lombardo-Pellegrino[55]	*A. viridis*	Soil	Rabbit, guinea-pig, cat	Rabbit, dog, cat, horse
Nocard[13]	*A. farcinicus*	Chronic suppurative disease of cattle	Guinea-pig	
Rabe[56]	*A. canis*	Abscess and draining sinus in neck and forepaw of dog	Rabbit, guinea-pig	
Redaelli[57]	*A. sanfelicei*	Rat	Guinea-pig, rabbit, rat, dog	Fowl
Silberschmidt[58]	*A. caprae*	Lung of goat	Rabbit, guinea-pig	Mouse
Trolldenier[59]	Unnamed	Caseopurulent abscess of dog		
Cuttino and McCabe	*N. intracellularis, n. sp.*	Granulomatous lesion in lymph nodes and spleen	Guinea-pig, rat, mouse	Rabbit, fowl, frog, goldfish

* Named *A. leishmani* by the authors; later identified as *A. asteroides* by Foulerton.[24]
† Named *A. freeri* by the authors; later identified as *A. asteroides* by the authors.

genicity for all animals tested to nonpathogenicity for any laboratory animal. Here again, none of them reacted exactly like the organism under consideration.

Conclusions

A new disease entity in man was characterized clinically by an abdominal mass, malnutrition, and an afebrile course. The morphologic, cultural, and biochemical characteristics of the causative organism, when contrasted with those of other organisms, present significant differences in every instance, justifying the designation of this organism as a new species to which the name *Nocardia intracellularis, n. sp.,* has been given. This organism is assigned to the genus Nocardia, family Actinomycetaceae.

In man the disease produced by this organism is a pure form of granulomatous inflammation, characterized by phagocytosis of the pathogen by reticulo-endothelial cells and proliferation of these cells. In the spleen and lymph nodes the proliferation of macrophages is of such proportion as to displace completely the normal structure. There is equilibrium between the organism and its host cell to the extent that massive intracellular parasitism constitutes perhaps the most distinctive feature of the disease. Comparatively little necrosis occurs, and there is no response on the part of polymorphonuclear leukocytes and other inflammatory elements customarily found in the common infectious granulomas.

Nocardia intracellularis, n. sp., produces a nonlethal disease in guinea-pigs, rats, and mice. It produces no lesions in chickens, rabbits, frogs, and goldfish.

In the experimental animal, *Nocardia intracellularis, n. sp.,* provokes a response which

differs sharply from the reaction in man. This difference is to be found in comparatively extensive necrosis and relative paucity of intracellular organisms. The lesions heal rapidly in the experimental animal and are accompanied by the production of sensitivity of the tuberculin type.

We are indebted to Dr. Wiley D. Forbus for many helpful suggestions and advice offered throughout the course of this study and in the preparation of this manuscript. We are also indebted to Drs. F. A. Wolf and N. F. Conant for advice during the study of the organism.

REFERENCES

1. Doan, C. A., Sabin, F. R., and Forkner, C. E. The derivation of giant cells with especial reference to those of tuberculosis. *J. Exper. Med.*, 1930, **52**, suppl. 3, 89–111.
2. Fite, G. L. II. Leprosy: the pathology of experimental rat leprosy. *Nat. Inst. Health Bull.*, 1940, No. 173, 45–75.
3. Bergey, D. H. Manual of Determinative Bacteriology. The Williams & Wilkins Co., Baltimore, 1939, ed. 5, p. 849.
4. Stokes, W. R. A study of the group Actinomyces with the report of a pathogenic species for man. *Am. J. M. Sc.*, 1904, **128**, 861–875.
5. Waksman, S. A., and Henrici, A. T. The nomenclature and classification of the actinomycetes. *J. Bact.*, 1943, **46**, 337–341.
6. Craig, C. F. The branched form of the *Bacillus tuberculosis* in sputum. *J. Exper. Med.*, 1898, **3**, 363–370.
7. Soule, M. H., and McKinley, E. B. Cultivation of *B. leprae* with experimental lesions in monkeys. *Am. J. Trop. Med.*, 1932, **12**, 1–36. Further studies on experimental leprosy and cultivation of *Mycobacterium leprae*. *Ibid.*, 1932, **12**, 441–452.
8. Clegg, M. T. Some experiments on the cultivation of *Bacillus leprae*. *Philippine J. Sc.*, Sect. B, 1909, **4**, 77–79.
9. Duval, C. W. The cultivation of the leprosy bacillus and the experimental production of leprosy in the Japanese dancing mouse. *J. Exper. Med.*, 1910, **12**, 649–665.
10. Badger, L. F., Patrick, D. W., Fite, G. L., and Wolfe, D. I. Leprosy: two strains of acid-fast bacilli isolated from a case of human leprosy. A comparison with other strains of acid-fast organisms with particular reference to the Lleras bacillus. *Nat. Inst. Health Bull.*, 1940, No. 173, 1–44.
11. Johnson, H. W. Studies on johnin. V. Producing and standardizing a potent product. *Am. J. Vet. Research*, 1944, **5**, 320–328.
12. Hagan, W. A. Personal communication, June 16, 1947.
13. Nocard, E. Note sur la maladie des boeufs de la Guadeloupe connue sous le nom de Farcin. *Ann. Inst. Pasteur*, 1888, **2**, 293–302.
14. Gasperini, G. Versuche über das Genus "Actinomyces." *Centralbl. f. Bakt.*, 1894, **15**, 684–686.
15. Eppinger, H. Ueber eine neue, pathogene Cladothrix und eine durch sie hervorgerufene Pseudotuberculosis (cladothrichica). *Beitr. z. path. Anat. u. z. allg. Path.*, 1890, **9**, 287–328.
16. Henrici, A. T., and Gardner, E. L. The acid-fast actinomycetes with a report of a case from which a new species was isolated. *J. Infect. Dis.*, 1921, **28**, 232–248.
17. Birt, C., and Leishman, W. B. A new acid-fast Streptothrix, pathogenic to man and animals. *J. Hyg.*, 1902, **2**, 120–128.

18. Chalmers, A. J., and Christopherson, J. B. A Sudanese actinomycosis. *Ann. Trop. Med.*, 1916–17, **10**, 223–282.
19. Berestneff, N. Die Actinomykose und ihre Erreger. *Virchows Arch. f. path. Anat.*, 1898, **152**, 399–400. (Cited by Schabad [41] and Henrici and Gardner.[16])
20. Henrici, A. T. Molds, Yeasts, and Actinomycetes. John Wiley & Sons, Inc., New York, 1930, ed. 1, p. 249.
21. Davis, D. J. An acid-fast Streptothrix (Nocardia). *Arch. Int. Med.*, 1914, **14**, 1–7.
22. Vuillemin, P. Addendum to Jolly, R. Sur une adénite à Nocardia ayant simulé un bubon pesteux. *Rev. méd. de l'est.*, 1920, **48**, 42-43. (Cited by Dodge.[60])
23. Davis, D. J., and Garcia, O. Experimental study of a pathogenic acid-fast actinomycete (Nocardia). *Arch. Dermat. & Syph.*, 1923, **7**, 1–13.
24. Gordon, R. E., and Hagan, W. A. A study of some acid-fast actinomycetes from soil with special reference to pathogenicity for animals. *J. Infect. Dis.*, 1936, **59**, 200–206.
25. Gammel, J. A. Actinomycosis without granules. *Arch. Dermat. & Syph.*, 1934, **29**, 287–297.
26. Goldsworthy, N. E. Pulmonary actinomycosis caused by an acid-fast species of Actinomyces. *J. Path. & Bact.*, 1937, **45**, 17–27.
27. Kirby, W. M. M., and McNaught, J. B. Actinomycosis due to *Nocardia asteroides*. *Arch. Int. Med.*, 1946, **78**, 578–591.
28. Umbreit, W. W. Studies on the proactinomycetes. *J. Bact.*, 1939, **38**, 73–89.
29. Rosebury, T. The parasitic actinomycetes and other filamentous microorganisms of the mouth. *Bact. Rev.*, 1944, **8**, 189–223.
30. Henrici, A. T. Molds, Yeasts, and Actinomycetes. (Revised by Skinner, C. E., Emmons, C. W., and Tsuchiya, H. M.) John Wiley & Sons, Inc., New York, 1947, ed. 2, p. 353.
31. Forbus, W. D. The Nature and General Pathological Significance of Granulomatous Inflammation. C. C. Thomas, Springfield, Ill. (In press.)
32. Coppen-Jones, A. Ueber die Nomenklatur des sog. "Tuberkelbacillus." *Centralbl. f. Bakt.*, 1896, **20**, 393–395.
33. Claypole, E. J. On the classification of the streptothrices, particularly in their relation to bacteria. *J. Exper. Med.*, 1913, **17**, 99–116.
34. Foulerton, A. G. R. The Milroy lectures on the streptotrichoses and tuberculosis. *Lancet*, 1910, **1**, 551–556; 626–631; 769–773.
35. Sanfelice, F. Streptothrix-Pseudotuberculose. *Centralbl. f. Bakt.*, 1905, **38**, 30–41.
36. Aoyama, T., and Miyamoto, S. Ueber die menschenpathogene Streptothrix. *Mitt. a. d. med. Fakult. d. k. Univ. zu Tokio*, 1898–1900, **4**, 231–276.
37. Buchholz, H. Ueber menschenpathogene Streptothrix. Ein Beitrag zur Aetiologie des acuten Lungenzerfalls. *Ztschr. f. Hyg. u. Infektionskr.*, 1897, **24**, 470–487.
38. Horst, A. Ein Fall von Streptothrixpyämie beim Menschen. *Ztschr. f. Heilk.*, 1903, **24**, 157–176.
39. Loehlein, M. Ueber Gehirnabszess durch Streptothrix. *München. med. Wchnschr.*, 1907, **54**, 1523–1525.
40. MacCallum, W. G. On the life history of *Actinomyces asteroides*. *Centralbl. f. Bakt.*, 1902, **31**, 529–547.
41. Schabad, J. A. Actinomycosis atypica pseudotuberculosa. *Ztschr. f. Hyg. u. Infektionskr.*, 1904, **47**, 41–80.

42. Musgrave, W. E., and Clegg, M. T. The etiology of mycetoma; report of a case of the ochroid variety occurring in the Philippine Islands and caused by a new species of Streptothrix (*Streptothrix freeri*). *Philippine J. Sc.*, Sect. B., 1907, **2**, 477–511.
43. Sabrazès, J., and Rivière, P. Les parasites du genre Streptothrix dans la pathologie humaine. *Semaine méd.*, 1895, **15**, 383. (Cited by Schabad[41] and Dodge.[60])
44. Butterfield, E. E. A case of pulmonary infection with an acid-fast actinomyces. *J. Infect. Dis.*, 1905, **2**, 421–430.
45. DeKorté, W. E. *Nocardia cylindracea:* a South African otomycosis. *Ann. Trop. Med.*, 1917–18, **11**, 265–278.
46. Flexner, S. Pseudo-tuberculosis hominis streptothricha. *J. Exper. Med.*, 1898, **3**, 435–450.
47. Gibson, A. G. A new pathogenic form of Streptothrix. (Abstract.) *J. Path. & Bact.*, 1919–20, **23**, 357–358.
48. Greco, N. V. Primer caso de pie de maduro o micetoma en la república Argentina. Tesis, Buenos Aires, 1904. (Cited by Dodge.[60])
49. Ferré, J., and Faguet, E. Sur un abcès du cerveau à Streptothrix. *Semaine méd.*, 1895, **15**, 359. (Cited by Foulerton,[84] Dodge,[60] and others.)
50. Bernstein, J. M. A fatal case of streptotrichosis with primary lesion in the lungs—the organism pathogenic for animals. *Proc. Roy. Soc. Med.* (Path. Sect.), 1908–09, **2**, 271–282.
51. Ljubimoff. Personal communication to Berestneff,[19] cited by Schabad.[41]
52. Scheele and Petruschky. Culturen und Präparate einer menschen-pathogenen Streptothrix-Art (Diagnose *in vivo*). *Verhandl. d. Kong. f. inn. Med.*, 1897, **15**, 550–553.
53. Ribytkof and Maloletkof. (Cited by Henrici and Gardner.[16])
54. De Mello, F., and St. Antonio Fernandes, J. F. Révision des champignons appartenant au genre Nocardia. *Mem. Asiatic Soc. Bengal*, 1918–23, **7**, 103–138. (Cited by Dodge.[60])
55. Lombardo-Pellegrino, P. Di una Streptothrix isolata del sotosuolo. *Riforma med.*, 1903, **19**,, 1065–1068. (Cited by Dodge.[60])
56. Rabe, C. Über einen neuentkeckten, pathogen Mikroorganismus bei dem Hunde. *Berl. thierärztl. Wchnschr.*, 1888, **4**, 65–68; 78–79.
57. Redaelli, P. Studii sulla nocardiasi sperimentale, actinomicosi, streptotricosi; infezione primaria, basi anatomiche e fattore cellulare della immunita naturale. *Boll. d. Ist. sieroterap. milanese*, 1928, **7**, 75–101; 121–135.
58. Silberschmidt. Sur un nouveau Streptothrix pathogène (*Streptothrix caprae*). *Ann. Inst. Pasteur*, 1899, **13**, 841–853.
59. Trolldenier. Ueber eine bei einem Hunde gefundene pathogene Streptothrix. *Ztschr. f. Thiermed.*, 1903, **7**, 81–109. (Cited by Dodge.[60])
60. Dodge, C. W. Medical Mycology. C. V. Mosby Co., St. Louis, 1935, pp. 694–785.

[*Illustrations follow*]

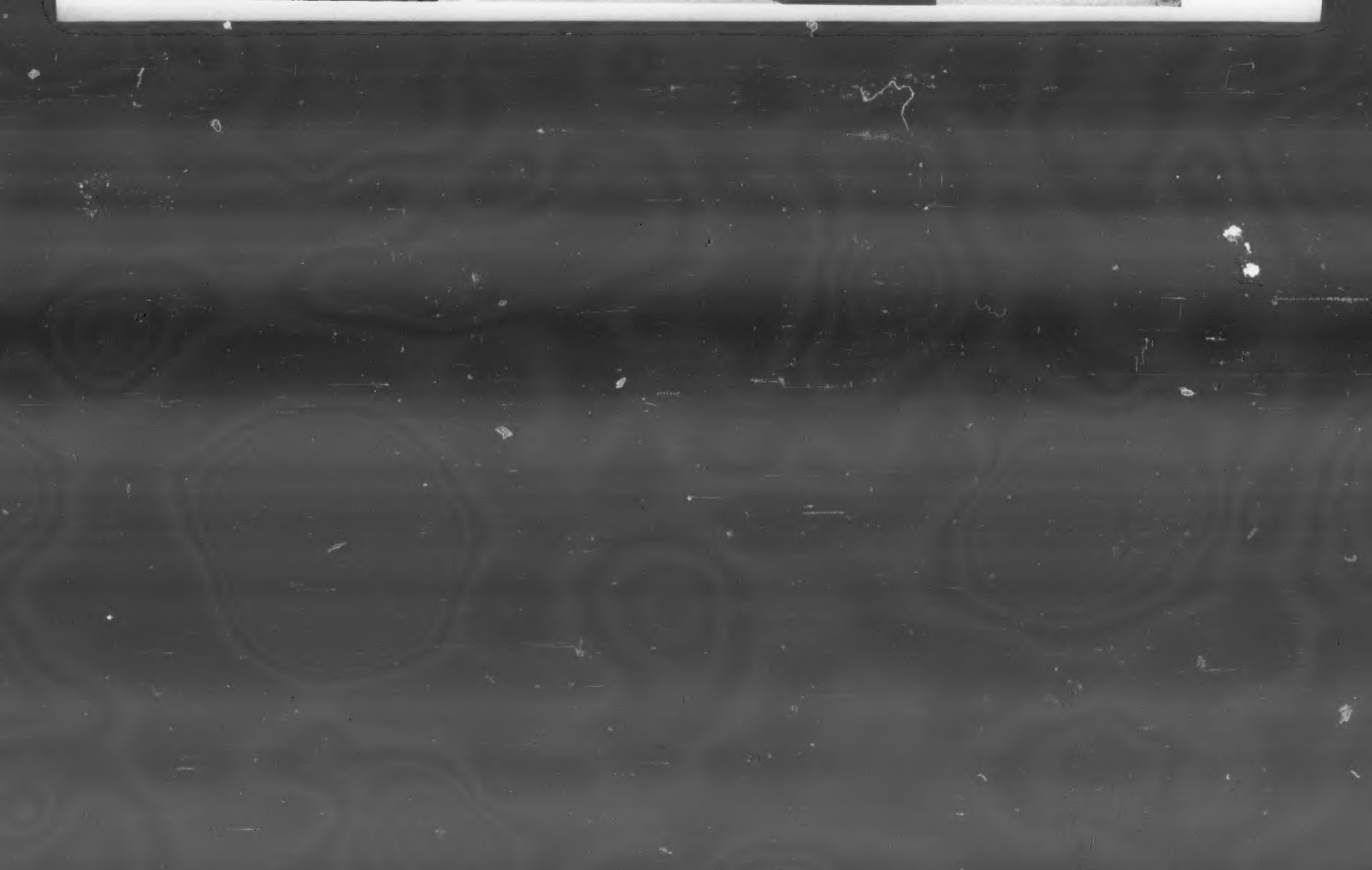
DESCRIPTION OF PLATES

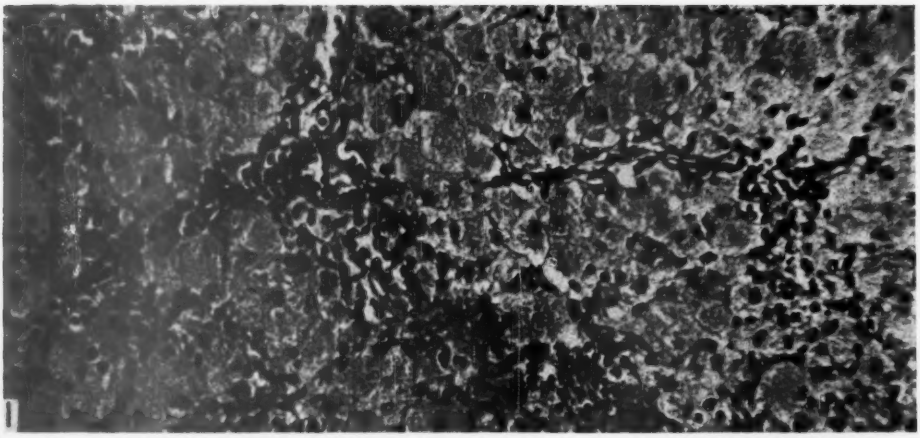
PLATE 1

FIG. 1. From the original lymph node, removed for biopsy, showing widespread replacement of lymphoid tissue by large foamy macrophages. Hematoxylin and eosin stain. × 290.

FIG. 2. Thoracic and abdominal viscera, showing the mass of enlarged and matted lymph nodes of the mesenteric and periaortic groups. There is also enlargement of the left subclavian nodes. The spleen, partially visible at the right, likewise is enlarged. The discoloration of the liver and lungs is an artifact.

FIG. 3. Colon showing large, irregular ulcers with necrotic bases. The intervening mucosa has lost its normal corrugation. The marginal lymph nodes show enlargement due to proliferation of macrophages.





Cuttino and McCabe

Granulomatous Nocardiosis

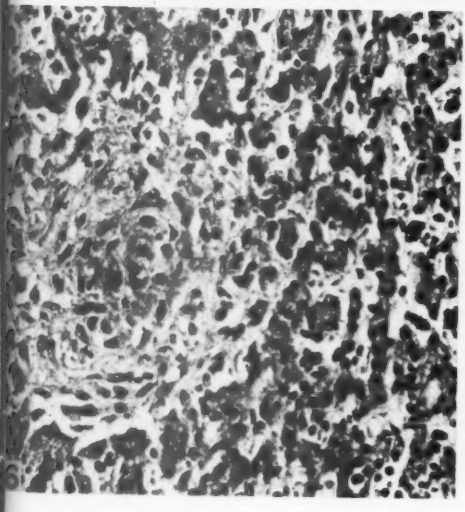
PLATE 2

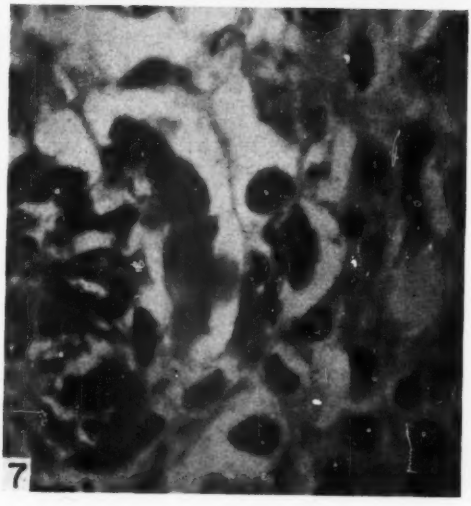
FIG. 4. Spleen with complete replacement of its structure by macrophages. The large, deeply stained bodies are multinucleated giant cells. Hematoxylin and eosin stain. × 40.

FIG. 5. Mesenteric lymph node with the same changes as were found in the spleen (Fig. 4). In both lymph node and spleen there is absence of necrosis, but the formation of giant cells is more impressive in the lymph node. Hematoxylin and eosin stain. × 40.

FIG. 6. A tuberculoid nodule in the liver. There is also enlargement of Kupffer cells. Hematoxylin and eosin stain. × 300.

FIG. 7. A higher power view of the same nodule seen in Figure 6. It is stained to show the great number of intracellular, acid-fast organisms, both in cells of the nodule and in isolated Kupffer cells. Ziehl-Neelsen's stain. × 725.





Cuttino and McCabe

Granulomatous Nocardiosis

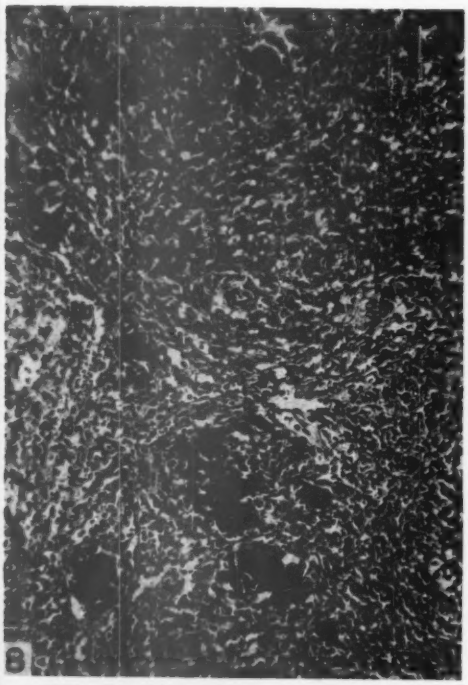
PLATE 3

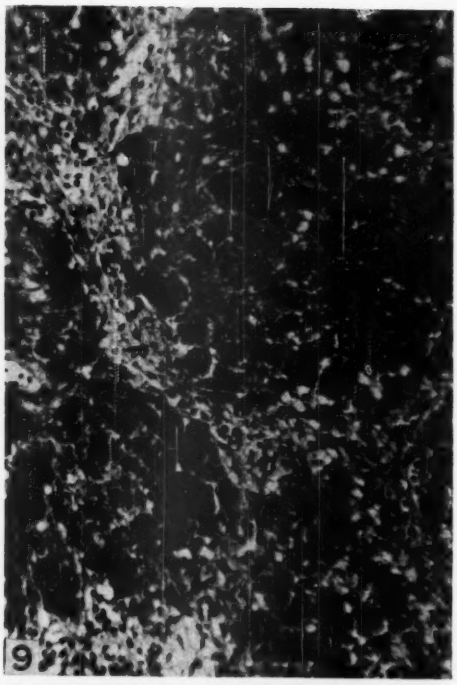
FIG. 8. Spleen showing multinucleated giant cells and concentrically arranged epithelioid cells about the central arteries of replaced malpighian corpuscles. Hematoxylin and eosin stain. × 140.

FIG. 9. Lymph node showing large mononuclear macrophages and giant cells. Some giant cells have clear, central cytoplasm surrounded by a foamy, peripheral zone. The displacement of normal lymphoid structure is illustrated. Hematoxylin and eosin stain. × 140.

FIG. 10. Spleen showing massive, acid-fast, intracellular parasitism of both large mononuclear macrophages and multinucleated giant cells. The absence of organisms in the interstices is a characteristic feature. Ziehl-Neelsen's stain. × 1400.

FIG. 11. Lymph node showing two of the giant cells seen in Figure 9. The clear, central zone results from absence of organisms, while in the periphery of the cells they are found in a radiating configuration. The extent of intracellular parasitism by acid-fast organisms is evident. Ziehl-Neelsen's stain. × 750.


8


9


10


11

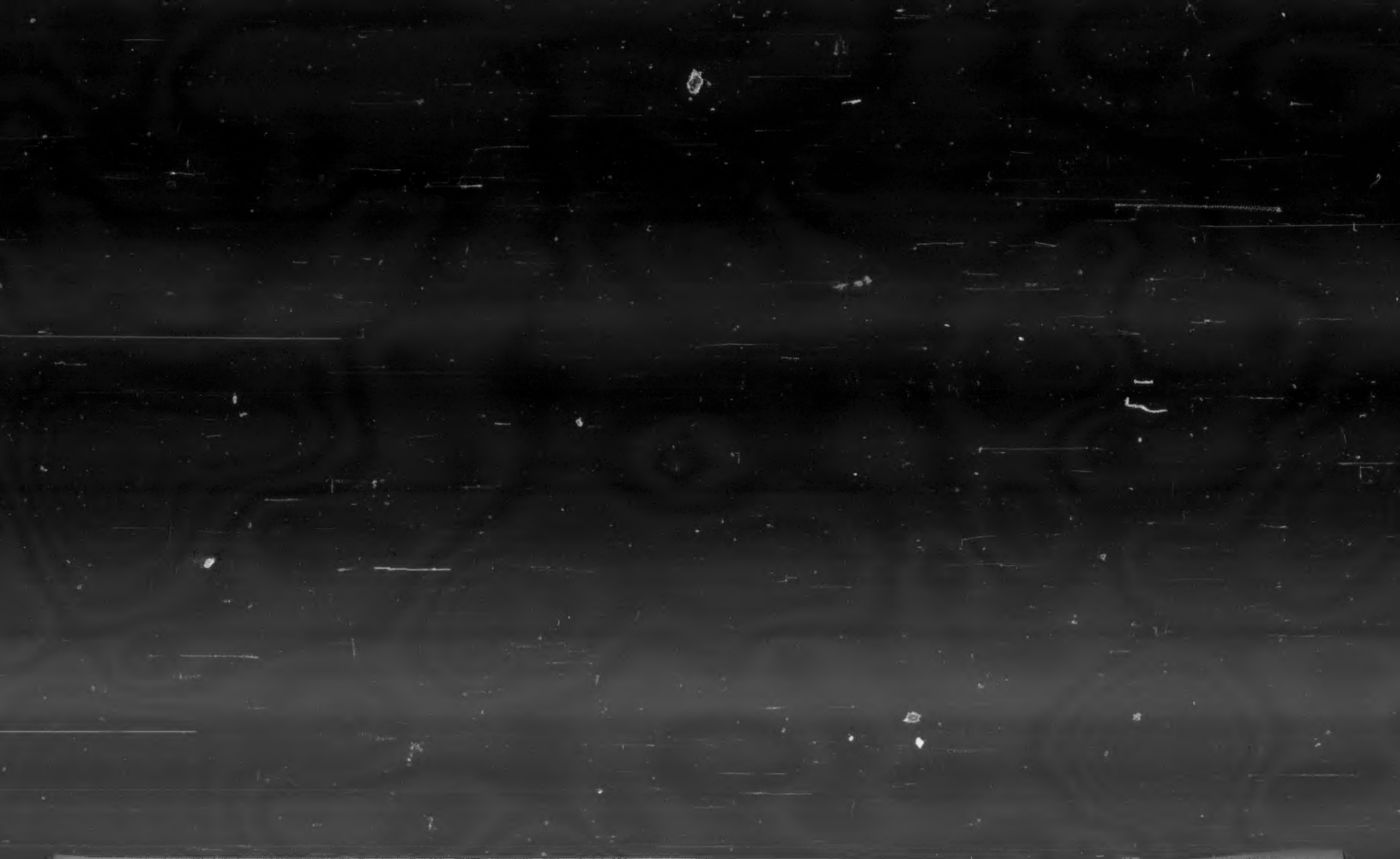
PLATE 4

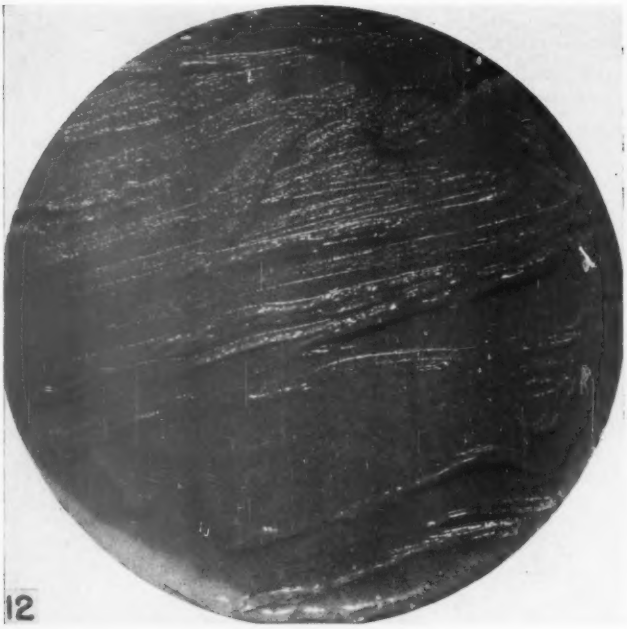
FIG. 12. A 10-day-old streaked plate of the causative organism on glycerin-egg medium, showing the smooth, confluent, moist growth without aerial mycelia.

FIG. 13. A 6-months-old culture on Sabouraud's slant illustrates the vermiform contour of the growth.

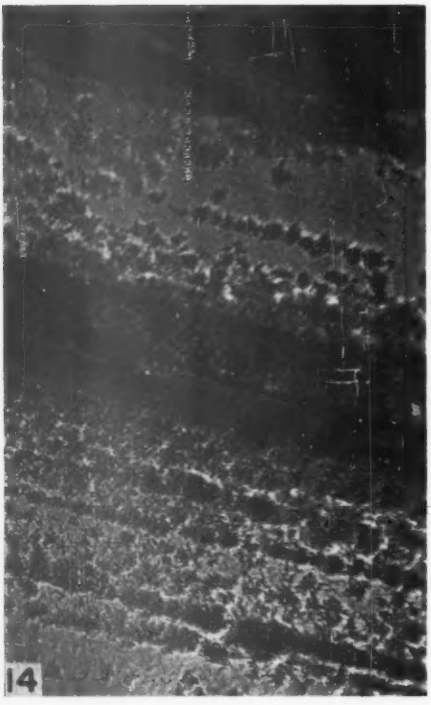
FIG. 14. A magnified view of a 12-day-old streak culture on Sabouraud's agar to show the details of colony growth. The individual colonies tend to coalesce and produce the smooth growth seen grossly in Figure 12. × 200.

FIG. 15. A stained smear of the organism transferred by loop from Sabouraud's agar slant (Fig. 13). The pleomorphic forms result from the fragmented hyphae. Gram's stain. × 1350.




12


13


14


15

PLATE 5

FIG. 16. A photomicrograph of the unstained organism growing on Sabouraud's agar in a Van Tieghem cell mount, showing mycelial growth with true branching. × 800.

FIG. 17. A stained smear of the organism from a 10-day-old litmus milk culture, with preservation of true branching and regularly spaced, spherical to ovoid bodies identified as arthrospores. Kinyoun's acid-fast stain. × 1700.

FIG. 18. Another view of the smear of the organisms from the 10-day-old litmus milk culture (Fig. 17), showing matting of hyphae. At the periphery of the mass may be seen the large pleomorphic, irregularly arranged, nonacid-fast bodies termed granules. Kinyoun's acid-fast stain. × 1350.

FIG. 19. A high-power view of a lymph node of a rat to show the scattered acid-fast organisms in a collection of large mononuclear macrophages. At left is a "Y" form (arrow). Ziehl-Neelsen's stain. × 1700.

Cuttino and McCabe

Granulomatous Nocardiosis

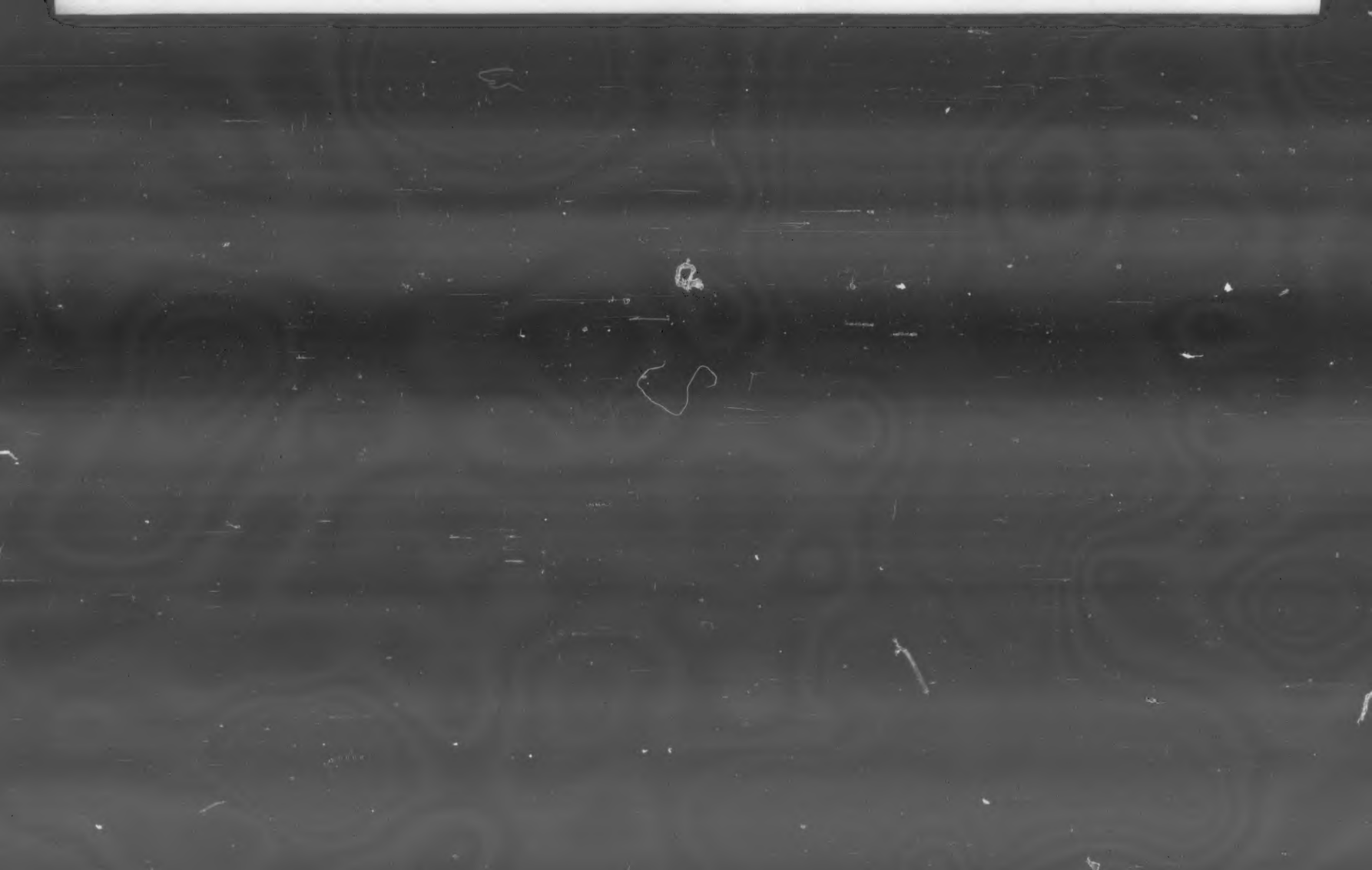
PLATE 6

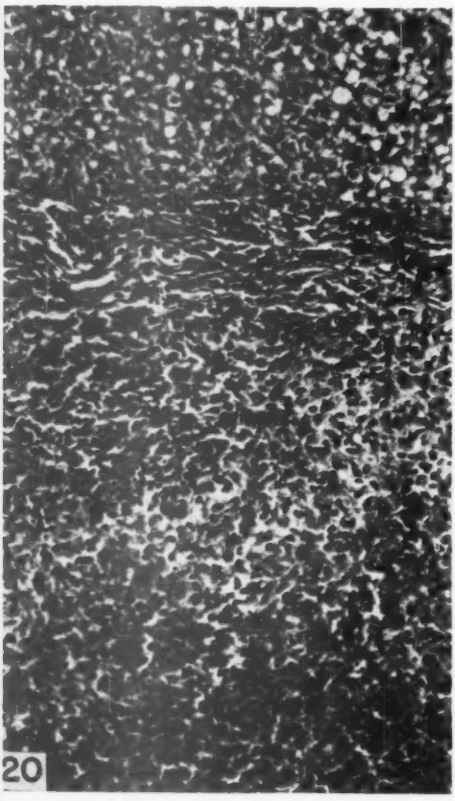
FIG. 20. Liver of a guinea-pig 4 weeks after subcutaneous injection of viable organisms. In the zone of necrosis (lower), organisms were found in clumps; in the zone of macrophagic proliferation (center), a few isolated intracellular parasites were found. There is a zone of fibrosis separating the lesion from normal liver tissue (above). Hematoxylin and eosin stain. × 150.

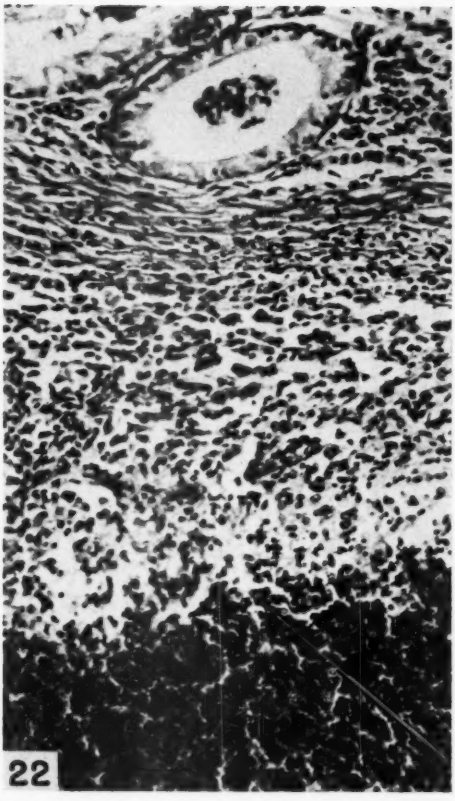
FIG. 21. Lungs of a guinea-pig showing enlargement of lymphoid follicles. In only one such instance were organisms stained within these follicles. Hematoxylin and eosin stain. × 150.

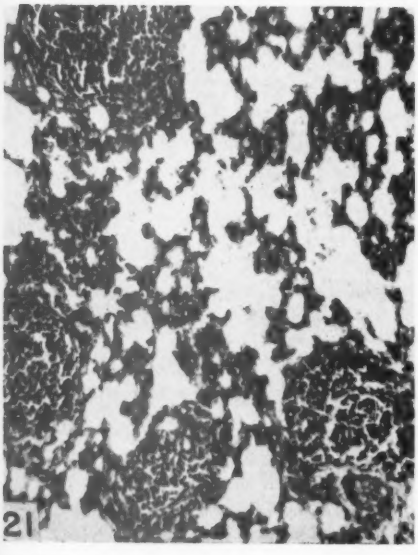
FIG. 22. Epididymis of a guinea-pig with necrosis in a central zone surrounded by macrophages as in Figure 20. Many acid-fast organisms were found in clumps in the necrotic tissue. They are fewer in number among the macrophages. Hematoxylin and eosin stain. × 200.

FIG. 23. Two mice showing sites of inoculation after 24 hours (right) and 7 days (left). The 24-hour lesion is a small papule seen at the arrow. The lesion after 7 days is a large ulcer.









Cuttino and McCabe

Granulomatous Nocardiosis

NONLIPID RETICULO-ENDOTHELIOSIS: LETTERER-SIWE'S DISEASE

A Report of Three Cases *

Ethelbred L. Schafer, M.D. †

(From the Institute of Pathology, Western Reserve University, and Hospitals, Cleveland, Ohio)

A clinicopathologic syndrome involving principally the reticulo-endothelial system in infants and young children and having a fatal outcome has come to be known as Letterer-Siwe's disease since the publication of an article by Abt and Denenholz [1] in 1936. In 1942 Green and Farber [2] postulated a possible relationship between Letterer-Siwe's disease, Schüller-Christian's disease, and eosinophilic granuloma of bone. A more general awareness of the disease and its possible relationship to Schüller-Christian's disease and eosinophilic granuloma should stimulate the reporting of additional cases of diseases involving the reticulo-endothelial system. This will aid in the clarification of these disease entities, if indeed they are such, or will serve to classify them properly as variants of a single disease process. With this in mind, three cases of Letterer-Siwe's disease autopsied at this Institute since November, 1940, are reported in detail. These cases fulfill the requirements for Letterer-Siwe's disease as formulated by Abt and Denenholz, namely: splenomegaly, hepatomegaly, generalized lymphadenopathy, localized tumors either over or in bone, secondary anemia, generalized hyperplasia of the cells composing the reticulo-endothelial system, a nonfamilial disease of infants terminating fatally.

Report of Cases

Case 1

A 4½-months-old white male infant was admitted to Babies and Childrens Hospital, Cleveland, on November 9, 1940, because of "white patches on the gums and tonsils and a lump on the right side of the head." Except for a cutaneous eruption stated to be present from birth, which had never completely disappeared, the infant was well until the age of 2½ months, when he developed anorexia, "colic," and an otitis media which required bilateral myringotomy. Examination on both occasions revealed cutaneous petechiae and enlarged tonsils. At 4 months, multiple buccal ulcers and a small lump in the right temporal region were noted. The parents and one sibling were alive and well.

Examination on admission revealed a well nourished infant. Over the trunk were many cutaneous lesions, petechiae, and yellow to dark-red papules, many less than 1 mm. in diameter (Fig. 1). Covering the scalp were small, yellow, crusted lesions. In the right temporal region was a subcutaneous, soft mass measuring 1.5 cm. in maximum diameter. Two shallow ulcers were found on the alveolar process of the maxilla. The tonsils were covered by a pale-gray exudate. The liver was palpable 2.5 cm. below the costal margin in the right midclavicular line. Several

* Received for publication, March 3, 1948.

† Now at Jackson Clinic, 16 S. Henry St., Madison 3, Wis.

defects in the skull were palpable through the scalp: one, measuring 1 by 1.5 cm., was beneath the mass in the right temporal region; a slightly larger one was present in the right occipital region; and a third was present in the right parietal region.

Laboratory Examinations. Blood: red blood cell count, 5,600,000; hemoglobin, 94 per cent (Sahli); white blood cell count, 12,000, with 78 polymorphonuclear leukocytes, 20 small lymphocytes, and 2 mononuclear cells per 100 white blood cells; serum cholesterol, 132 mg.; cholesterol esters, 75 mg.; total fat, 656 mg.; and lecithin, 195 mg. per 100 cc. Urine showed a trace of albumin, with 2 or 3 white blood cells per low-power field. Roentgenograms of the skull showed three irregularly rounded, radiolucent areas as follows: One, 1.5 cm. in diameter, in the anterior and inferior portion of the right parietal bone; another, 1 cm. in diameter, in the same bone near the lamboidal suture; and a third, 3 cm. in diameter, in the occipital bone (Fig. 2). The long bones, ribs, and pelvis showed no abnormalities roentgenologically except an area of lessened density at the proximal end of the right humerus on the medial aspect, 0.1 by 0.5 cm. in size. Roentgenograms of the chest showed a peculiar fine "honey combing" of the entire lung fields and a mottled appearance of each apex to the level of the 4th rib.

During the 8 days the infant was in the hospital the temperature remained below 38°C. except on the first day when it rose to 38.9°C. Dyspnea which was noted on the day of admission became progressively worse and was not relieved by administration of intranasal oxygen. On November 16, subcutaneous emphysema of the neck and upper thorax was observed and the infant died later that day. The clinical diagnosis was Schüller-Christian's disease and bilateral chronic suppurative otitis media.

Autopsy (no. 7184) was performed by Dr. F. M. Barry, 3 hours after death.

Gross Description. The body was that of a white male infant weighing 6,250 gm. Inspection of the skin and mucous membranes and palpation of the skull and subcutaneous tissues of the neck confirmed the presence of cutaneous and buccal lesions, osseous defects, and emphysema as described previously. The thymus was of normal size, its cut surfaces were lobulated, pale-brown mottled with yellow and flecked with firm, circumscribed, white foci measuring 2 to 3 mm. in diameter. The thyroid weighed 5 gm. The heart weighed 41 gm. (expected weight, 29 gm.) and, except for hypertrophy, was grossly normal. The lungs were pale-pink mottled with gray. On their surfaces were many bullae measuring from 0.1 to 2 cm. in diameter. These had thin, transparent walls and contained air. One along the upper border of the left lung was ruptured and probably was the source of the mediastinal and subcutaneous emphysema. Air spaces measuring 0.1 to 2 cm. in diameter were surrounded by firm septa which were pale pinkish tan mottled with yellow, and measured 0.2 to 0.5 cm. in width, imparting a "honey comb" appearance to the pulmonary parenchyma. The larynx, trachea, and bronchi were normal. The liver weighed 275 gm. (expected weight, 188 gm.), and was grossly normal. The gastro-intestinal tract showed no abnormalities except that the lymphoid tissue of the small and large

intestines was more prominent than usual. The solitary follicles measured up to 0.1 cm. in diameter. The pancreas, adrenals, kidneys, urinary bladder, prostate, and testes were all normally located and showed no lesions grossly. The spleen weighed 30 gm. (expected weight, 16 gm.). Gray follicles, 1 mm. in diameter, surrounded by dark-red pulp were seen on the cut surface. All the lymph nodes throughout the body were enlarged, measuring from 0.5 to 1.5 cm. in diameter. They were pale yellow and firmer than is normal. Their cut surfaces were homogenous and lacked their usual pattern. The brain, which weighed 750 gm. (expected weight, 420 gm.), was grossly normal. The skull bones varied greatly in thickness, in some places being less than 2 mm. There were three osseous defects with smoothly rounded margins, occupying positions as shown by the roentgenograms (Fig. 2). These defects were composed of firm, reddish brown tissue overlying but not adherent to the dura. There was no apparent abnormality of the bones of the orbit, sella turcica, or base of the skull. There was a small perforation in each tympanic membrane. The bone marrow of the sternum, ribs, and vertebrae was reddish brown and of normal consistency.

The microscopic observations on this case and the succeeding two cases will be described together following the gross observations on the third case. The similarity between the lesions of the various organs can be more effectively depicted in this manner.

Case 2

This 8-months-old male infant was seen by a pediatrician 1 week before admission to Babies and Childrens Hospital on June 20, 1945, because of fever and "sores on the gums." The liver and spleen were palpable and a blood count showed moderate leukocytosis. A tentative diagnosis of leukemia was made. The parents stated that at birth the infant had had petechiae over the body and hands which disappeared with the administration of vitamin K. Five weeks before the present illness, a light brown, crusted and scaling eruption appeared over the body and petechiae were noted on the hands, face, and trunk. The infant had been listless for several weeks, he cried when handled, his breathing was more rapid, and his skin paler than usual. The parents and 3 siblings were alive and well.

On admission the child appeared acutely and chronically ill; temperature, 39.9°C.; pulse, 148; and respirations, 60. The skin and mucous membranes were pallid and many petechiae were present over the trunk, being most numerous on the upper back and back of the head (Fig. 3), but not present on the arms, legs, or feet. Several bullous lesions, 2 cm. in diameter, and containing clear, pale-yellow fluid, were present on the buttocks and inner aspects of the thighs. Elsewhere the skin was dry and scaling. The anterior maxillary, cervical, and axillary lymph nodes were palpable, firm, and discrete. The liver was palpable 4 cm. below the right costal margin and the spleen, 4 cm. below the left costal margin.

Admission Laboratory Examinations. Blood: Red blood cell count, 2,400,000; hemoglobin, 38 per cent (Sahli); white blood cell count, 20,500; platelet count, 450,000; 77 polymorphonuclear leukocytes, 20 lymphocytes, 2 mononuclear cells,

and 1 eosinophil per 100 white blood cells; bleeding time, 4 minutes 15 seconds; clotting time, 1 minute 45 seconds; prothrombin time, 80 seconds (normal, 30 seconds); serum protein, 6.1 gm.; cholesterol, 100 mg.; cholesterol esters, 36 mg.; total fat, 716 mg.; lecithin, 209 mg.; calcium, 7.3 mg.; phosphorus, 3.4 mg.; nonprotein nitrogen, 35 mg. per 100 cc. of blood; culture, no growth after 6 days; Wassermann and heterophile antibody agglutination tests, negative. Urine: normal. Roentgenograms of the skull and extremities showed no osseous defects. Roentgenograms of the chest showed a widened supracardiac shadow, with increased hilar bronchovascular markings. A splenic puncture showed atypical mononuclear cells suggestive of xanthomatosis (lipidosis).

Throughout his hospital course the patient was acutely ill. Penicillin and sulfadiazine were given without effect on the patient's fever, which showed daily elevations from 38° to 40°C. Supportive measures, consisting of transfusions, hykinone, and ascorbic acid, were used without permanent improvement. The red blood cell count and hemoglobin increased slightly as a result of repeated transfusions, but there was a progressive decrease in the number of white blood cells. The patient lost weight rapidly, with marked wasting of the extremities and edema of the neck and head. He died on the 22nd hospital day. The clinical diagnosis was lipidosis, possible Niemann-Pick's or Gaucher's disease.

Autopsy (no. 8862) was performed by Dr. G. S. Wilson, 6½ hours after death.

Gross Description. The body was that of a poorly nourished, white male child weighing 11 kg. The cutaneous lesions of the body and scalp were as described in the clinical abstract. There was hemorrhage into the right leaf of the diaphragm and 60 cc. of blood-tinged fluid in the peritoneum. The thymus, which weighed 13 gm. (expected weight, 18 gm.), was lobulated, reddish brown, and firm. The cut surfaces were reddish brown as compared to the normal pale pinkish or grayish tan. The heart weighed 53 gm. (expected weight, 37 gm.) and, except for size, was normal in all respects. The lungs were uniformly grayish pink and well expanded, but crepitation was decreased throughout. The liver weighed 550 gm. (expected weight, 254 gm.). Sections revealed nonbulging, reddish brown surfaces upon which the lobular pattern was faintly discernible. The esophagus, stomach, small and large intestines, pancreas, adrenals, kidneys, urinary bladder, prostate, and testes were all normally located and not remarkable grossly. The spleen was markedly enlarged, weighing 200 gm. (expected weight, 20 gm.). It was firmer than average, and sections made against increased resistance revealed surfaces which were brownish red flecked with multiple scattered, pale brownish gray foci measuring up to 3 and 4 mm. in diameter. An accessory spleen, measuring 1 cm. in diameter, resembled the spleen in color and consistency. The cervical, axillary, mediastinal, suprapancreatic, mesenteric, and para-aortic lymph nodes were firm, discrete, and enlarged to approximately three times their normal size. Cut surfaces were flat, firm, and brownish red mottled with gray. The brain

weighed 780 gm. (expected weight, 714 gm.) and was grossly normal. Examination of the skeletal system showed no osseous lesions. The marrow from the sternum, ribs, and lumbar vertebrae was of average consistency and pale brownish red. The skull bones were normal. The middle ear and mastoid air cells showed no evidence of inflammation.

Case 3

A white male infant was admitted to Babies and Childrens Hospital on December 20, 1945, at the age of 3 weeks because of diarrhea, vomiting, and fever. The infant was born at term of a 23-year-old primipara who was well during her pregnancy. A generalized cutaneous eruption had been present at birth. This was described by a pediatrician as a "polymorphous rash consisting of petechiae, red macules and pale tan scaly crusts." During the first week the eruption cleared spontaneously except for small crusted lesions of the scalp. The infant vomited his feedings frequently and had gained weight slowly. Three days before admission he became irritable and a hoarse cry developed; the following day he had 10 to 12 watery, pale yellow stools. One day before admission a generalized cutaneous eruption again appeared, beginning as petechiae over the lower extremities and progressively spreading over the trunk and becoming macular and papular.

On admission the infant appeared neither acutely nor chronically ill but his cry was hoarse. There was a generalized papular and macular eruption over the entire body except the face, palms of the hands, and soles of the feet. Petechiae were scattered among the other lesions. Yellow crusted lesions were present in the scalp, and red macules on the hard palate. Lymph nodes, except the inguinal, were not palpable; these were small, firm, and discrete. The abdomen was distended and tympanitic but the liver and spleen could be palpated, the liver 5 cm. below the right costal margin, the spleen 4 cm. below the left costal margin.

Laboratory Examinations. Blood: Red blood cell count, 3,900,000; hemoglobin, 82 per cent (Sahli); white blood cell count, 6,600, with 50 neutrophilic polymorphonuclear leukocytes, 46 lymphocytes, 2 mononuclear cells, and 2 eosinophils per 100 cells; platelets were present in normal numbers; serum cholesterol, 150 mg.; total fat, 484 mg.; lecithin, 274 mg. per 100 cc. Urine: normal. Roentgenograms of the skull and long bones showed no osseous defects; the lungs were normal. A blood culture taken on the 18th hospital day showed no growth after 6 days.

The patient was given sulfadiazine, 0.5 gm. on admission and 0.125 gm. every 4 hours thereafter for 15 days. He was given Ringer's solution by mouth and parenteral fluids until the diarrhea was under control on the 2nd hospital day. The temperature rose to 39.5°C. on the 2nd and 3rd hospital days, then fell to normal. Diarrhea reappeared on the 8th hospital day and could not be controlled by the usual methods. The weight, which had increased to 3,250 gm., fell to 2,800 gm. and remained there even after marked edema appeared on the 18th hospital day. The lesions of the palate progressed to form confluent, shallow ulcers with gray, firm margins and bleeding bases. On the 19th hospital day the red blood cell count was 1,300,000; the hemoglobin, 38 per cent; white blood cell count, 9,000. Many of the lymphocytes in the blood smear had vacuolated cytoplasm and several blast forms contained vacuoles. The patient died on the 20th hospital day. The clinical diagnosis was reticulo-endotheliosis, probably Letterer-Siwe's disease.

Autopsy (no. 9042) was performed by Dr. A. H. Salans, 3½ hours after death.

Gross Description. The body was that of an emaciated white male infant weighing 2.8 kg. Inspection revealed cutaneous and buccal lesions, and edema as noted in the clinical history. The thymus weighed 4.5 gm. (expected weight, 10 gm.). The external and cut surfaces were firm, lobulated, and yellow brown mottled with purplish red. The thyroid weighed 1 gm. and was normal grossly. The heart weighed 17.5 gm. (expected weight, 12 gm.). The right ventricle was slightly dilated; otherwise the heart was normal. The right and left lungs weighed 32 and 26 gm. respectively and, except for slightly reduced crepitation, were normal grossly. The liver weighed 133 gm. (expected weight, 80 gm.). The cut surfaces were nonbulging, deep purple, and without definite markings. The Peyer's patches of the ileum and the solitary lymphoid follicles of the terminal ileum and colon were more prominent than expected. In the latter situation they measured up to 0.2 cm. in diameter and had umbilicated centers. The remainder of the gastrointestinal system was normal grossly. The adrenals and genito-urinary system showed no gross abnormalities. The spleen weighed 22.5 gm. (expected weight, 8 gm.). Cut surfaces showed faintly visible, pale gray follicles surrounded by deep reddish purple tissue. The mesenteric lymph nodes were enlarged, measuring up to 1.5 cm. in diameter. Cut surfaces were pale pink, mottled with gray, circular foci averaging 1 mm. in diameter. The peribronchial and mediastinal lymph nodes were not remarkable. Bone marrow from the lumbar vertebrae was pinkish brown mottled with gray. The autopsy permit did not include examination of the long bones, skull, and brain.

MICROSCOPIC OBSERVATIONS

The characteristic cutaneous lesions showed collections of large atypical mononuclear cells and small hemorrhages in the corium. There was parakeratosis with scale formation (Fig. 4) and atrophy of overlying epithelium. A few polymorphonuclear leukocytes were present in the epidermis and corium. In case 2, scaling was not a prominent feature (Fig. 5). The cutaneous lesions in case 3 showed sparsely scattered eosinophils in the corium and atypical mononuclear cells in the subcutaneous fat, in addition to the above changes.

Sections of the thymus showed no recognizable thymic tissue in cases 1 and 2. In case 3 a rare Hassall's corpuscle could be identified. Traversing the gland and dividing it into lobules were narrow bands of fibrous tissue and intermingled fibroblasts. The substance of the gland was composed of loosely arranged cells of several different types. Relatively large cells, approximately 12 to 20 μ, were predominant in all

cases. These were of irregular outline, with acidophilic cytoplasm and centrally or eccentrically placed vesicular nuclei, which varied in shape, some being oval, other lobulated and grooved, and still others irregularly "crumpled." Chromatin was irregularly distributed in the nuclei and no nucleoli were noted except in case 3. Many of the atypical cells in this case contained large violaceous nucleoli. Mitotic figures were infrequent in each case. The cytoplasm of some of the atypical cells in cases 1 and 2 was vacuolated. There were scattered foci of large multinucleated giant cells having 4 to 12 nuclei and irregular cell borders. Many showed phagocytosis of brown pigment, erythrocytes, and polymorphonuclear leukocytes (Fig. 6). Multinucleated forms were smaller and less numerous in cases 2 and 3. Scattered eosinophils and foci of small lymphocytes were present in each thymus. Portions of each gland were unusually vascular and capillary proliferation was conspicuous. Rare foci of necrosis, approximately one-sixth the diameter of a low-power field, were seen in case 2. Special stains revealed sudanotropic droplets in the cytoplasm of some of the large mononuclear and giant cells, but examination of the tissues under crossed Nicol prisms showed no doubly refractile crystals. Sections treated with hydrochloric acid and ferrocyanide showed a moderate amount of iron pigment within phagocytic cells.

In only the first case did the thyroid show involvement by characteristic lesions composed of large atypical mononuclear cells. Foci of these cells formed nodules in the interlobular connective tissue and in some instances projected into acini.

Sections of the heart and aorta were normal in all cases except the first, which showed small hemorrhages in the myocardium and endocardium.

The amount of pulmonary tissue involved by reticulo-endothelial cells and the degree of bronchopneumonia varied in the three cases. In case 1 the changes were predominantly interstitial. Alveolar walls were thickened by virtue of an increase in the cellular constituents. These cells were principally of the type previously described. Atypical mononuclear cells were free also within alveoli; an occasional cell contained sudanotropic droplets. A few lymphocytes and neutrophilic and eosinophilic polymorphonuclear leukocytes were admixed with the atypical mononuclear cells. Interlobular septa, peribronchial and perivascular tissue, and included portions of the pleura contained similar cells in variable numbers. Some were observed within lumina of blood vessels. Emphysema was conspicuous in some sections and there was a small focus of bronchopneumonia in the left lower lobe. In case 2 the

interstitial changes, while similar, were less profound than in the previous case. There was a small focus of bronchopneumonia in the right middle lobe. The last case showed the least interstitial involvement by large atypical mononuclear cells. A section from the right upper lobe showed a nodule composed chiefly of these cells (Fig. 7). Sections of the larynx and trachea in this case showed many fibroblasts in the subepithelial connective tissue and scattered foci of polymorphonuclear cells.

Except for diffuse cloudy swelling of hepatic cells and fatty metamorphosis, changes in the liver in case 1 were slight. A few Kupffer cells were enlarged and there was a slight increase of connective tissue in the portal regions. A small nodule of atypical mononuclear cells was present within the wall of a bile duct. In cases 2 and 3, many Kupffer cells were enlarged and showed phagocytosis of polymorphonuclear leukocytes and erythrocytes. The connective tissue of the portal regions was greatly increased and contained many large atypical mononuclear cells, some of these forming nodules of 12 to 16 cells. Foci of hematopoiesis were numerous in case 3. Eosinophils not confined to these foci numbered 12 to 15 per high-power field. Several foci of atypical mononuclear cells were present in the interlobular connective tissue of the pancreas in the first and second case.

A section taken through the gingival ulcer in the first case showed a layer of large atypical mononuclear cells of the type seen elsewhere, covered by neutrophilic polymorphonuclear leukocytes mixed with fibrin. The atypical cells were grouped in nodules. Some of the cells contained mitotic figures and some bizarre nuclei (Fig. 8). Sections from the remaining portions of the gastro-intestinal tract in each case differed only in degree of involvement of the interglandular and lymphoid tissue by atypical cells. This was less in the stomach and jejunum. The lymphoid follicles and Peyer's patches were partially replaced by large mononuclear cells (Fig. 9). Mitotic figures were observed in every 2 or 3 high-power fields. Eosinophils numbered 6 to 10 per high-power field. These changes were most profound in case 3 in which the mucosa of the jejunum, ileum, and colon was increased two to three times its normal thickness because of the large number of atypical cells in the interglandular stroma. The mucosa over the Peyer's patches and lymphoid follicles was ulcerated.

Diffusely scattered throughout the loose peripelvic connective tissue of the kidneys were large atypical mononuclear cells. In addition there was a small circumscribed nodule of similar cells in the cortex of the right kidney in case 2. In this case sections from the testes showed numerous atypical cells in the connective tissue underlying the tunica

vaginalis and in the interlobular septa; in the latter situation they occasionally formed nodules of 15 to 20 cells. The testes in the other two cases showed no abnormal changes.

In case 3 the medullary cells of the adrenal were replaced mostly by atypical mononuclear cells mixed with a few eosinophils. Similar cells were present in the pericapsular fat and some of these exhibited phagocytosis of erythrocytes.

In cases 2 and 3 the normal pattern of the spleen was considerably altered by the presence of large numbers of atypical mononuclear cells. In case 1 the general pattern was retained even though nodules composed of 12 to 30 large mononuclear cells formed the centers of most of the follicles (Fig. 10). In all cases there was endothelial hyperplasia of central arterioles and sinuses. Phagocytosis of erythrocytes and of brown pigment was conspicuous, particularly in cases 1 and 2.

Sections from many lymph nodes in all cases were similar but varied considerably in degree of involvement. In case 1 the general microscopic appearance was similar to that in the thymus except that giant multinucleated cells were scarce. In case 2 the normal pattern was more or less well preserved, with an increase of large mononuclear cells in the central portions and peripheral sinuses. Phagocytosis of polymorphonuclear leukocytes and erythrocytes by the reticulo-endothelial cells in the para-aortic lymph nodes was conspicuous. Many of these cells had vacuolated cytoplasm (Fig. 11). In case 3, eosinophils were relatively numerous. One lymph node from the mesenteric group contained several minute abscesses surrounded by mononuclear cells. In the tonsils of case 1 the lymphoid tissue was replaced by nodules of atypical mononuclear cells, which penetrated deeply into the underlying tissue and in some foci extended into striated muscle. No sudanotropic droplets were demonstrated in the reticulo-endothelial cells of the spleen, lymph nodes, or tonsils in case 1, but they were present in the vacuolated cells of the para-aortic lymph nodes in case 2 and in scattered mononuclear cells in lymph nodes, pulmonary alveoli, and cutaneous lesions in case 3.

Special stains showed inconstant amounts of reticulin where the reticulo-endothelial cells were most numerous. In some instances fibrils of reticulin were in intimate association with these cells. In other sites a small amount of reticulin surrounded foci or nodules of these cells in the spleen and lymph nodes but the fibrils did not extend among the cells. None was observed in association with the large mononuclear cells of the cutis in case 3, yet there was a heavy reticulin network in the pulmonary nodule composed of these cells in the same case.

A section through one of the cranial defects in case 1 revealed dense

fibrous connective tissue replacing the bone. Foci of cells, similar to those described as reticulo-endothelial elsewhere, were present. Adjacent to this defect the usual two tables of compact bone were present but the bone marrow contained many foci of large, irregularly shaped reticulo-endothelial cells. Bone marrow from the sternum in case 2 and from a lumbar vertebra in case 3 showed a few large atypical mononuclear cells among the normal cellular components.

The anatomic diagnoses in case 1 were: Reticulo-endotheliosis (nonlipid) of skin, gums, thymus, thyroid, lungs, liver, pancreas, lymphoid tissue of gastro-intestinal tract, pelvis of kidneys, spleen, lymph nodes, tonsils, bones of skull, and bone marrow; bronchopneumonia of lower lobe of left lung; emphysema of lungs; emphysema of tissues of anterior mediastinum and subcutaneous tissues of face, neck, and upper anterior thorax; fatty metamorphosis of liver; perforations of tympanic membranes (recent bilateral myringotomy).

The anatomic diagnoses in case 2 were: Nonlipid reticulo-endotheliosis (Letterer-Siwe's disease) involving skin, thymus, lungs, liver, pancreas, lymphoid tissue of intestines, kidneys and peripelvic fat, testes, pars nervosa of pituitary body, spleen, lymph nodes, and bone marrow; bronchopneumonia of middle lobe of right lung; recent hemorrhage into right leaf of diaphragm; hemoperitoneum.

The anatomic diagnoses in case 3 were: Nonlipid reticulo-endotheliosis (Letterer-Siwe's disease) involving skin, thymus, larynx, liver, lymphoid tissue of intestines, spleen, lymph nodes, bone marrow, adrenals, and periadrenal and subcutaneous fat; bronchopneumonia; hematopoiesis of liver; edema of peri-orbital tissues, hands, and feet.

Comments

The remarkable similarity of the clinical courses and of the anatomic and microscopic observations in the foregoing cases leaves no doubt that they belong in the category of reticulo-endotheliosis known as Letterer-Siwe's disease. All showed a cutaneous eruption, pronounced hepatomegaly and splenomegaly, and variable degrees of lymphadenopathy at the time of hospitalization. Other presenting symptoms (mass over the right temporal bone in case 1, buccal ulcers in cases 1 and 2, diarrhea and hoarseness in case 3) may all be explained by localized proliferation of reticulo-endothelial cells. The course of the disease in all three instances was rapid and accompanied by a progressive anemia in cases 2 and 3. Only on admission was a blood count done on case 1, and this showed no anemia, but anemia probably did develop later. The fatal termination of these cases is in accord with other reports of this

TABLE I

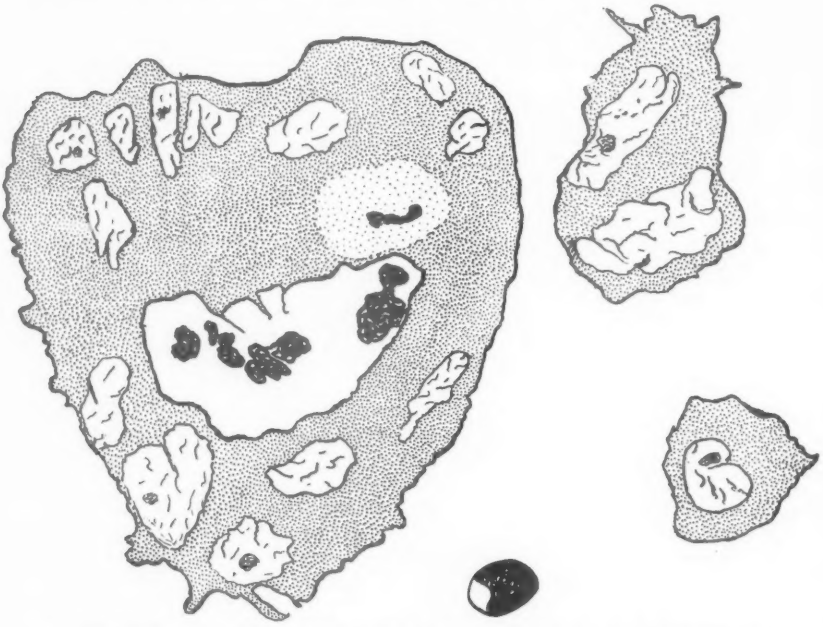
Summary of Pertinent Observations on the Clinical Course, Physical and Laboratory Examination

	Case 1	Case 2	Case 3
Sex and race	Male, Gentile	Male, Gentile	Male, Gentile
Age	4½ months	8 months	3 weeks
Presenting symptom	"Lump," right side of head	Pallor, "buccal ulcers"	Diarrhea
Fever	38.9°C.	39.9°C.	39.5°C.
Liver	Palpable	Palpable	Palpable
Spleen	Not palpable	Palpable	Palpable
Lymph nodes	Not palpable	Axillary, cervical, maxillary: palpable	Inguinal: palpable
Cutaneous eruption	From birth	Petechiae at birth	Polymorphous rash at birth
Osseous lesions	Multiple defects in skull, defect in proximal end of right humerus	None by x-ray	None by x-ray
Red blood cell count	5,600,000 (hemoglobin, 94%)	2,400,000 (hemoglobin, 38%)	3,900,000 (hemoglobin, 82%)
White blood cell count	12,000	20,500	6,600
Platelets	Not recorded	450,000	Normal numbers
Serum cholesterol	132 mg./100 cc.	100–135 mg./100 cc.	150 mg./100 cc.
Serum lecithin	195 mg./100 cc.	206 mg./100 cc.	274 mg./100 cc.
Total fats	656 mg./100 cc.	716 mg./100 cc.	484 mg./100 cc.
Duration	Approximately 2 months	3–4 weeks	23 days

condition. Table I summarizes the pertinent observations relative to the clinical courses, physical and laboratory examinations.

In all cases the anatomic and morphologic features were similar. There was universal involvement of the cells of the reticulo-endothelial system. The marked proliferation or hyperplasia of these cells resulted in an increase in the size of those organs having a conspicuous reticulo-endothelial component. This hyperplasia was both diffuse and focal. In the latter instance it resulted in the formation of nodules. While the individual cells varied slightly in size from case to case and sometimes from organ to organ in the same case, they were strikingly uniform in other respects. Text-Figure 1 is a camera lucida drawing of typical cells from the thymus in case 1, which shows some of the salient characters of these cells. Text-Figure 2 is

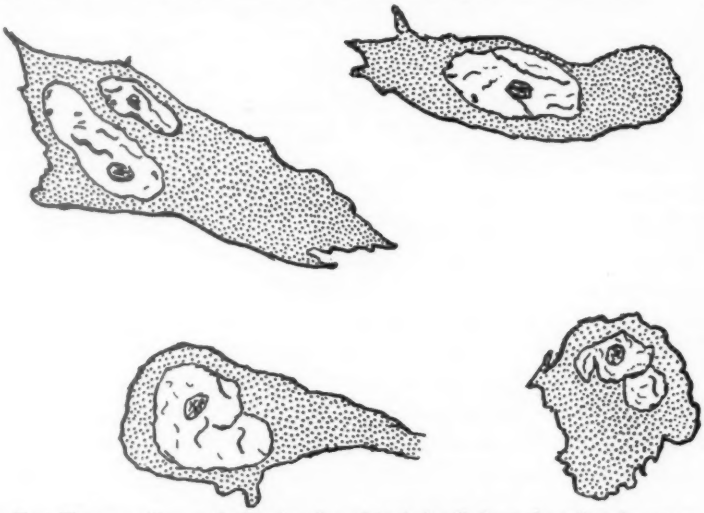
a camera lucida drawing of typical cells seen in the spleen in the same case. Another noteworthy feature was the endothelial and fibroblastic proliferation observed in some of the granulomatous lesions of certain organs, principally, the thymus, lungs, and spleen.



Text-Figure 1. Camera lucida drawing of typical cells seen in the thymus in case 1. The giant cell contains phagocytized leukocytes. A clear halo surrounds the nuclear remnants of several of these cells. The small cell with a dark nucleus in lower center field is a lymphocyte, for comparison of the relative size of the cells.

Even though cutaneous lesions differed slightly on gross examination in the three cases, the microscopic appearance was strikingly similar (Figs. 4 and 5). Few organs escaped involvement by reticulo-endothelial hyperplasia. The thymus showed the most striking changes, being unrecognizable on microscopic examination alone in cases 1 and 2. The significance of these constant and profound changes is not known. The lungs, liver, spleen, and lymphoid tissue were constantly but variably involved. The lungs showed the most profound changes in case 1. The degree of hepatic involvement varied considerably, and microscopically did not seem sufficient to cause hepatomegaly. Splenic involvement resulted in splenomegaly. In case 2, the spleen was ten times the expected size. The lymph nodes and the lymphoid tissue of the gastro-intestinal tract showed similar changes. This involvement did not result

in marked lymphadenopathy, nor in unusual increase in size of the solitary follicles and Peyer's patches of the intestines except in case 3. The endocrine glands were not universally involved by reticulo-endothelial proliferation. The only glands so involved were the thyroid in case 1, the testes and pituitary body in case 2, and the adrenals in case 3. The bone marrow showed slight to moderate involvement in all instances, with groups of reticulo-endothelial cells replacing the normal cells of the bone marrow. This change, if extensive, may be a factor in the production of anemia of the progressive nonregenerative type which occurs in Letterer-Siwe's disease. Only in case 1 did the changes produce osseous defects, visible on roentgenographic examination.



Text-Figure 2. Camera lucida drawing of typical cells from the spleen in case 1. Of note are the irregular cell outline and large vesicular nuclei.

While phagocytic activity of the reticulo-endothelial cells was observed in all cases, it was particularly profound in cases 1 and 3. This property of the reticulo-endothelial cells might explain the presence of lipid in some of the cells. In case 3 the phagocytic activity was concerned chiefly with the ingestion of erythrocytes. If pronounced, this activity may be an additional factor in the production of anemia. Another noteworthy microscopic feature was the presence of mitotic figures in the foci of hyperplasia. This observation has not been uniformly reported.

Review of Literature

In 1936 Abt and Denenholz [1] reported a case of nonlipid reticulo-endotheliosis in an infant, which fulfilled the criteria established by

Siwe [3] in 1933. The first case was reported by Letterer,[4] in 1924, as proliferative aleukemic reticulosis. The second and third cases were those of Akiba [5] and Guizetti [6] reported in 1926 and 1931. Siwe reported the fourth case and reviewed the 3 previous cases. Abt and Denenholz included in their series the cases of Podvinec and Terplan [7] (1931), Gittins' [8] case 4 (1933), and the cases of Foot and Olcott [9] (1934), and of Roussy and Oberling [10] (1934). These cases are ably summarized by them [1] (Table 2, page 506). A case by van Creveld and Ter Poorten,[11] reported in 1935, was not accepted as a noninfectious reticulo-endotheliosis because the infant had had an otitis media from the age of 4 weeks. This case had defects of the membranous bones of the skull and cystic lesions of the humeri and ribs. Abt and Denenholz also excluded a case by Schultz, Wermbter, and Puhl.[12] Siwe believed this to be an atypical case of Schüller-Christian's disease and it was similarly classified by Abt and Denenholz.

In September, 1940, Wallgren [13] reported 2 cases and reviewed 15 cases. He included those reported by Abt and Denenholz [1] and added several which had been reported as infectious nonlipid reticulo-endotheliosis. The cases of Schultz, Wermbter, and Puhl [12] (1924), Krahn [14] (1926), Sherman [15] (1929), Gittins' case 2 [8] (1933), Uher [16] (1933), Klostermeyer [17] (1934), and of van Creveld and Ter Poorten [11] (1935) were regarded by Wallgren as infectious nonlipid reticulo-endotheliosis. The cases of Podvinec and Terplan,[7] and of Foot and Olcott,[9] cited by Abt and Denenholz as noninfectious nonlipid reticulo-endotheliosis, were thought by Wallgren to be infectious reticulo-endotheliosis. It is interesting to note that Akiba [5] regarded his own case as being related to an infection, yet both Wallgren, and Abt and Denenholz classified it as a noninfectious reticulo-endotheliosis. Similarly, Letterer,[4] Guizetti,[6] Podvinec and Terplan,[7] and Foot and Olcott [9] thought that possibly infection was an etiologic factor in their cases. These cases, 17 in all, were summarized by Wallgren and will not be reviewed further here except to state that Sherman's case [15] probably does not belong in this category. This case, which showed marked hyperplasia of the reticulo-endothelial system without granulomas, occurred in an 11-day-old infant that had erysipelas, streptococcal septicemia, and jaundice. Klostermeyer's case [17] is regarded as a lymphoid leukemia by several investigators.

Paige [18] presented 2 previously unreported cases of Letterer-Siwe's disease in 1935. Between 1936 and 1940 no additional cases appeared in the literature. A critical review reveals a number of cases, appearing under various titles, which probably belong to this clinicopathologic

syndrome when the accepted criteria are applied. At least one such case, brought to my attention by Paige,[19] was reported as Hodgkin's disease in an infant by Wollstein and McLean[20] in 1926. This case has been reviewed and in retrospect is thought to be a case of Letterer-Siwe's disease. In 1944 Jaffe and Lichtenstein[21] reported a case in an article on eosinophilic granuloma.

Early in 1940 Glanzmann[22] reported a case of Abt-Letterer-Siwe's disease in a 2-year-old female. Some features of this case, namely, osseous defects of the skull, exophthalmos, and presence of lipid in some of the granulomatous lesions, caused him to think that the condition was closely related to the xanthomatosis of Schüller-Christian's disease. Glanzmann regarded the case of Schultz, Wermbter, and Puhl[12] as a transitional form between Letterer-Siwe's and Schüller-Christian's diseases. Wallgren's[13] second case also presented some features of Schüller-Christian's disease (lipid deposit in macrophages and osseous defects of the skull). He postulated that there was an overlapping of the two diseases and that the rapidity of the course of the disease determined the amount of lipid deposited in the macrophages.

In an article on eosinophilic granuloma, Gross and Jacox[23] reported a case of Letterer-Siwe's disease which occurred in a 13-months-old child who died 5 months after onset of symptoms. At autopsy the lungs were thought to resemble those seen in Schüller-Christian's disease, but otherwise the clinico-anatomic complex was similar to that of Letterer-Siwe's disease. Gross and Jacox cited a case reported by Erber[24] in 1931 which they thought belonged in the category of reticulo-endothelial diseases. Siwe[3] did not include this case in his original series nor was it included by Abt and Denenholz[1] or by Wallgren[13] in their series. Lipid was described in some granulomatous lesions in this case and it was probably excluded by the above investigators for that reason.

A number of other cases displaying features of the two diseases and thus thought to represent transitional forms have been reported by Freund and Ripps[25] (1941), Galeotti Flori and Parenti[26] (1937), Freud, Grossman, and Dragutsky[27] (1941), and Sweitzer, Winer, and Cumming[28] (1939). Freund and Ripps reported a case as Schüller-Christian's disease which 14 months before death showed marked proliferation of reticulum cells and formation of syncytial reticulum giant cells in a lymph node taken for biopsy. A very small amount of lipid was present in the reticulum cells, but the diagnosis of Schüller-Christian's disease could not be made until the autopsy material was examined. Characteristic granulomatous lesions with numerous foam cells were found in the lungs and paravertebral fascia, but the lymph nodes

showed changes similar to those seen at biopsy. In their case a mass had appeared in the left groin at the age of 7 weeks but growth and development were normal until the age of 7 months, when pallor and anemia were noted. Later, areas of rarefaction in the skull were demonstrated by roentgenograms. The child died at the age of 2 years following perforation of a gangrenous appendix. The disease in this case was unusually protracted and supports Wallgren's [13] contention that the presence of lipid in the macrophages of granulomatous lesions is related to the duration of the disease. Freund and Ripps cited a similar case reported by Galeotti Flori and Parenti. Biopsy of a lymph node, 1 year before death of a 20-months-old child, showed marked reticuloendothelial hyperplasia, and the characteristic cells were devoid of lipid. At autopsy, typical lipid granulomas of Schüller-Christian's disease were found.

Freud, Grossman, and Dragutsky [27] reported a case of "acute idiopathic cholesterol granulomatosis," which in many respects was similar to Letterer-Siwe's disease. It occurred in a 7-months-old boy and was rapidly fatal (3 months). The observations at autopsy were similar to described cases of Letterer-Siwe's disease except that there was a large amount of lipid in the involved organs, namely, the thymus, lungs, liver, spleen, and lymph nodes. These investigators accepted their case as supporting Wallgren's [13] tenet that Letterer-Siwe's and Schüller-Christian's diseases are basically the same. The lipid content in this instance was greater than would be expected in a disease existing only 3 months.

Another example of a borderline case is given by Sweitzer, Winer, and Cumming.[28] A 3-year-old boy was well until the age of 2½ years when he complained of pain and weakness in the back and left leg, which limited his activities. Shortly thereafter cutaneous lesions of papular character appeared and anemia developed. Biopsy of the skin showed obliteration of the rete pegs with numerous nonlipid reticuloendothelial cells in the cutis. Biopsy of the left femur showed many large mononuclear cells, with abundant, finely granular, but only slightly vacuolated cytoplasm and multinucleated giant cells. At autopsy 45 days later many organs were involved with granulomatous lesions which resembled the nodules in the biopsy material.

Merritt and Paige [29] reported a case in which there was marked cutaneous involvement in a child, 3 years of age. Other symptoms had appeared approximately 1½ years earlier. At autopsy there was extensive hyperplasia of the reticulo-endothelial cells without evidence of lipid deposition in the lymph nodes and spleen, while cells of similar

lesions in the thymus, lungs, dura, and bones contained large amounts of lipid and were thought to be typical of Schüller-Christian's disease.

Another case of an intermediate type is that recorded by Grady and Stewart [30] in 1934. The disease occurred in a 3-year-old female who had been ill for approximately 2 years with "running ears." Of particular interest were the lesions in the spleen, some lymph nodes, and portions of the bone marrow, which resembled the granulomas of nonlipid reticulo-endotheliosis. Elsewhere, including the mastoid processes, the lesions were typical of Schüller-Christian's disease.

If the broader concept introduced by Glanzmann [22] and Wallgren [18] is accepted, at least one case (case 1), reported by Lane and Smith [31] in 1939 as "Schüller-Christian disease with cutaneous lesions," can be considered a transitional form bridging the gap between nonlipid (Letterer-Siwe's disease) and lipid reticulo-endotheliosis (Schüller-Christian's disease).

Jaffe and Lichtenstein [21] went further in an attempt to correlate nonlipid reticulo-endotheliosis (Letterer-Siwe's disease) with other diseases involving the reticulo-endothelial system, *i.e.*, idiopathic xanthomatosis (Schüller-Christian's disease) and eosinophilic granuloma. They believed that the different clinico-anatomic observations merely represent varying gradations of severity in the same process—Letterer-Siwe's disease showing the most severe and acute manifestations and being rapidly fatal, with little or no lipid deposition occurring in the reticulo-endothelial cells; Schüller-Christian's disease representing a chronic form of the same disease with a protracted course and the presence of typical foam cells in the lesions which have not been replaced by collagenous connective tissue; and eosinophilic granuloma being the most benign form of the disease with recovery usually occurring. Green and Farber,[2] and Farber [32] had previously suggested that the same basic disorder was responsible for the three diseases.

Several reported cases of reticulo-endotheliosis in adults, variously called aleukemic reticulosis, aleukemic monocytic leukosis, systemic reticulo-endotheliosis, are stated by the authors to resemble reticulo-endotheliosis in infants. Such a case was reported in an adult female, 36 years of age, by Ritchie and Meyer [33] in 1936. Goldzieher and Hornick [34] reported a case in a 75-year-old male and reviewed 22 similar cases appearing in the literature prior to 1931. Not all of these occurred in adults, as he included Akiba's [5] and Letterer's [4] cases. Dameshek's [35] case was in a 51-year-old man. Dameshek stressed the uniformity of the changes in the reticulo-endothelial system irrespective of age and cited cases that occurred from 11 days (Sherman [15]) to 75 years

of age (Goldzieher and Hornick [34]). As yet, there is no convincing evidence that the reticulo-endotheliosis occurring in adults is the same disorder as the infantile form under discussion.

Discussion

There has been considerable speculation and discussion concerning the etiology of Letterer-Siwe's disease. Siwe [3] divided into two categories the diseases of the reticulo-endothelial system not belonging to the then recognized xanthomatoses (Schüller-Christian's, Gaucher's, and Niemann-Pick's diseases). In the first group he included those cases associated with an acute infection known as infectious reticulo-endotheliosis; in the second group he placed those cases in which no significant infection was present, now known as Letterer-Siwe's disease. Both are regarded as nonlipid reticulo-endotheliosis. Because of the similarity of the histologic changes and the distribution of the characteristic lesions in infectious reticulo-endotheliosis and Letterer-Siwe's disease, Wallgren [13] justifiably challenged the belief that these are separate disease entities. He also presented arguments and evidence for believing that Schüller-Christian's disease is a chronic form of Letterer-Siwe's disease and that, as such, it should be removed from the xanthomatoses.

A critical analysis of the 3 cases reported in this paper indicates that Letterer-Siwe's disease is of noninfectious origin. In each instance, the cutaneous lesions, which I consider a most important and perhaps the earliest manifestation of the syndrome, were present at birth and antedated the fever. There is no reason to believe that an intra-uterine infection was present in any of the cases. Unfortunately, in none was the placenta examined, but there was nothing in the clinical course of the pregnancies to suggest prenatal infection. Case 1 had bilateral otitis media early in the course of the disease, but the possible involvement of the contiguous structures of the ear by reticulo-endothelial granulomas simulating an infectious process must be considered. Glatt,[36] Lichty,[37] and Grady and Stewart [30] described involvement of the middle ears and mastoid processes in Schüller-Christian's disease by granulomas which produced symptoms indistinguishable from chronic otitis media and mastoiditis. The disease was recognizable only after microscopic examination of material removed at operation or autopsy.

If fever is the criterion for infection, infection was present in all previously reported cases as well as in the three presented here, as temperatures above normal were recorded in all at some time during

the illness. In some the fever was of the Pel-Ebstein type often seen in Hodgkin's disease. An unexplained fever is also often present in leukoses. Since the etiology of these diseases is still unknown, no opinion can be expressed concerning the cause of the fever. Any disease, not primarily infectious, may be accompanied or complicated by concurrent or intercurrent infection, and this may occur also in Letterer-Siwe's disease.

Some anatomic features of these cases tend to support the hypothesis that Letterer-Siwe's and Schüller-Christian's diseases are variants of the same basic disorder. In case 1 there was roentgenographic evidence of skull defects similar to those described in Schüller-Christian's disease (Fig. 2). A number of cases have now been reported in which membranous bones of the skull were involved (Wallgren's [13] case 2, Glanzmann,[22] Abt and Denenholz,[1] and my case 1).

In all cases of the present series variable numbers of cells with vacuolated cytoplasm were found in the typical lesions in one or more sites, although in only one case (case 2) were they at all numerous. Microscopic examination of the spleen or para-aortic lymph nodes in case 2, without a study of additional sections from other organs, might have resulted in a diagnosis of Schüller-Christian's disease. This was also suggested by the appearance of the atypical cells obtained on splenic puncture. It is difficult to reconcile the presence of lipid-containing cells in these cases with Wallgren's hypothesis that the lipid deposit in the granulomatous lesions is a result of chronicity and occurs in the more slowly developing cases. This is especially true of case 3 which occurred in an infant who died at the age of 41 days. Obviously there are factors, other than duration of the disease, which determine lipid deposition. In all cases the thymus showed extensive fibroblastic proliferation and under conditions of chronicity may ultimately have undergone fibrosis and collagenization, features thought to be peculiar to Schüller-Christian's disease.

Another point of anatomic similarity between Letterer-Siwe's and Schüller-Christian's diseases is suggested by the occurrence of cutaneous lesions in approximately one-third of all cases of the latter (Rowland [38]) and in all reported cases of Letterer-Siwe's disease. All variations of the cutaneous eruptions, except the bronze pigmentation and lipid infiltration of the eyelids (xanthoma palpebrarum) described by Chester [39] and by Chester and Kugel [40] as occurring in Schüller-Christian's disease, have been noted in Letterer-Siwe's disease. Petechiae have been noted by all authors. Seborrheic eczema or seborrhea-like eczema was noted by Abt and Denenholz [1] and by Wallgren [13] (case 1).

Papular lesions were described by Wallgren in his case 2, and maculopapular lesions were observed in case 2 of the present series, as were also petechiae and bullous lesions, and a "polymorphous rash" in case 3.

Two or more conditions cannot be considered variants of the same pathologic process just because they are similar anatomically. They must also be similar clinically and have a common cause. All accepted cases of Letterer-Siwe's disease have occurred in infants and young children and have invariably been fatal. An estimated 30 per cent of the cases of Schüller-Christian's disease recover. Generally, Schüller-Christian's disease occurs in children and young adults, which may account for the different clinical outcome in about one-third of the cases. The rapidly fatal cases of Schüller-Christian's disease have occurred in children under 4 years of age. Some of these have shown inconstant amounts of lipid in the granulomatous lesions, and constitute borderline cases. The classical triad of Schüller-Christian's disease depends on the involvement of specific organs or structures by the granulomatous lesions and is encountered too infrequently to be of great value in establishing a diagnosis of that condition. It is not disconcerting that this triad has not been reported in Letterer-Siwe's disease. Glanzmann's [22] case, which had skull defects and unequal bilateral exophthalmos, might conceivably have developed diabetes insipidus if the course of the disease had been of longer duration.

No racial predominance has been noted in Letterer-Siwe's disease. When Rowland [38] reviewed a series of cases of Schüller-Christian's disease in 1928, he concluded that the disease occurred with greater frequency in members of the Jewish race. As increasing numbers of cases have been reported, this racial difference is no longer evident.[41]

The nature of the cells involved in both diseases has interested many investigators. That the principal cells are derived from the so-called reticulo-endothelial system is generally accepted. There is divergent opinion, however, concerning what causes these cells to proliferate. If the proliferation is neoplastic, more pleomorphism should be observed in the proliferating cells. These cells are remarkably uniform from case to case. The cells in the thymus showed the greatest variation. Gross and Jacox [23] reported pleomorphism of cells in the thymus and spleen of their case, suggestive of a sarcoma. The occurrence of mitotic figures in the three cases reported here probably represents rapid proliferation. If mitotic figures were more numerous and if they exhibited abnormal forms, neoplasia would be more tenable. As a rule the mitotic figures were infrequent in regions of reticulo-endothelial proliferation in the thymus, liver, and spleen. They were more common in the foci of re-

ticulo-endothelial cells associated with infection, *i.e.*, in lesions of the skin, tonsils, buccal ulcers, lymphoid tissue of small and large intestine. If, in the future, an infectious agent is implicated, the proliferation of reticulo-endothelial cells must be regarded as a hyperplastic response.

The relationship of eosinophilic granuloma to the two conditions considered above is not so clear although roentgenographically the osseous lesions in the three conditions are indistinguishable. Microscopically, the granulomas of eosinophilic granuloma bear a resemblance to the first two diseases. The most conspicuous constant feature is the presence of large atypical mononuclear cells, many exhibiting phagocytosis. Interspersed among these are variable numbers of eosinophils and giant cells. Foam cells may or may not be present and are usually in direct relation to the duration of the lesions. Clinically, the benign course and the regression and healing of the osseous lesions, either spontaneously or following curettage or radiation therapy, make it difficult to identify eosinophilic granuloma with Letterer-Siwe's and Schüller-Christian's diseases.

Otani and Erhlich [42] and Jaffe and Lichtenstein [21,43] reported lesions of eosinophilic granuloma occurring in long bones and skull. They emphasized the seemingly benign course of the disease. Green and Farber [2] suggested that visceral lesions probably do occur because eosinophils were found in a lymph node biopsy from one of their cases and they advised that a guarded prognosis should be given. Dundon, Williams, and Laipply [44] were of the same opinion. Curtis and Cawley [45] have reported a case of eosinophilic granuloma of bone with cutaneous manifestations of the disease verified by biopsy. Weinstein, Francis, and Sprofkin [46] recently have reported a case with multiple osseous lesions of eosinophilic granulomatous type. The case had roentgenographic evidence of pulmonary infiltration similar to that seen in Schüller-Christian's disease. The patient recovered from the osseous and pulmonary lesions.

Jaffe and Lichtenstein [21] stated that eosinophils are found only in the osseous lesions of Letterer-Siwe's disease. In all three of my cases, eosinophils were found in visceral as well as skeletal granulomas. Eosinophils also occur in the granulomatous lesions of Schüller-Christian's disease. Gross and Jacox,[23] in a review of 84 cases of Schüller-Christian's disease, found that eosinophils occurred in variable numbers in 29 cases. Fraser's [47] article on skeletal xanthomatosis contains several colored drawings of typical granulomas of Schüller-Christian's disease, which are indistinguishable from the lesions of eosinophilic granuloma. Thannhauser [48,49] contended that eosinophilic granuloma of the bone is

the "monosymptomatic form of a well known systemic granulomatous disorder [Schüller-Christian's disease] in which histiocytes, eosinophils and xanthoma cells are observed in the lesion at different phases." Jaffe and Lichtenstein [50] believed that it is necessary to make a clinical distinction between eosinophilic granuloma of bone and Schüller-Christian's and Letterer-Siwe's diseases even though morphologically they apparently represent different phases of the same basic disorder.

Conclusions

Including the 3 cases reported in this paper, the number of cases which meet the established criteria for Letterer-Siwe's disease does not exceed 24.

This study has not disclosed the cause of Letterer-Siwe's disease, but it does offer evidence that the disease is not initiated by infection. In all cases, the cutaneous lesions preceded by 2 to several weeks the manifestations of infection, *i.e.*, sore throat, otitis media, diarrhea, and fever. Infection must therefore be considered secondary or intercurrent, and not causative.

That Letterer-Siwe's and Schüller-Christian's diseases represent different manifestations of the same basic disorder of the reticulo-endothelial system seems probable from the number of borderline or transitional cases which have been reported. Twelve such cases were found in a review of the literature. That such a relationship does exist between these two diseases is accepted by Green and Farber,[2] Jaffe and Lichtenstein,[50] Mallory,[51] and Letterer,[52] as well as by Wallgren.[13]

That a possible relationship exists between the above two conditions and eosinophilic granuloma has been suggested by the same investigators. Thannhauser [48,49] believed that eosinophilic granuloma is actually a phase of the disorder known as Schüller-Christian's disease and as such should not be considered as a separate clinical entity.

Regardless of the morphologic similarity among the three diseases, the clinical course of eosinophilic granuloma varies so greatly from that of the other two conditions that a sharp clinical distinction between these disorders of the reticulo-endothelial system is justified until a common etiologic factor has been demonstrated for all.

I wish to acknowledge my indebtedness to Dr. C. F. McKhann of Babies and Childrens Hospital, University Hospital, Cleveland, Ohio, who kindly gave permission to use the clinical records of the three cases presented in this paper.

REFERENCES

1. Abt, A. F., and Denenholz, E. J. Letterer-Siwe's disease. Splenohepatomegaly associated with widespread hyperplasia of nonlipid-storing macrophages;

discussion of the so-called reticulo-endothelioses. *Am. J. Dis. Child.*, 1936, 51, 499–522.

2. Green, W. T., and Farber, S. "Eosinophilic or solitary granuloma" of bone. *J. Bone & Joint Surg.*, 1942, 24, 499–526.
3. Siwe, S. A. Die Reticuloendotheliose—ein neues Krankheitsbild unter den Hepatosplenomegalien. *Ztschr. f. Kinderh.*, 1933, 55, 212–247.
4. Letterer, E. Aleukämische Retikulose. Ein Beitrag zu den proliferativen Erkrankungen des Retikuloendothelialapparates. *Frankfurt. Ztschr. f. Path.*, 1924, 30, 377–394.
5. Akiba, R. Über Wucherung der Retikulo-Endothelien in Milz- und Lymphknoten und ihre Beziehung zu den leukämischen Erkrankungen. *Virchows Arch. f. path. Anat.*, 1926, 260, 262–270.
6. Guizetti, H. U. Zur Frage der infektiös bedingten Systemerkrankungen des reticuloendothelialen Apparates in Kindesalter. *Virchows Arch. f. path. Anat.*, 1931, 282, 194–208.
7. Podvinec, E., and Terplan, K. Zur Frage der sogenannten akuten aleukämischen Retikulose. *Arch f. Kinderh.*, 1931, 93, 40–55.
8. Gittins, R. Studies in the anaemias of infancy and early childhood. Part IX. Anaemia and reticulo-endotheliosis. *Arch. Dis. Childhood*, 1933, 8, 367–396.
9. Foot, N. C., and Olcott, C. T. Report of a case of nonlipoid histiocytosis (reticuloendotheliosis) with autopsy. *Am. J. Path.*, 1934, 10, 81–95.
10. Roussy, G., and Oberling, C. Akute, wahrscheinlich infektiöse aleukämische Retikulose bei einem Säugling. *Wien. med. Wchnschr.*, 1934, 84, 407–413.
11. van Creveld, S., and Ter Poorten, F. H. Infective reticulo-endotheliosis chiefly localized in lungs, bone marrow and thymus. *Arch. Dis. Childhood*, 1935, 10, 125–142.
12. Schultz, A., Wermbter, F., and Puhl, H. Eigentümliche granulomartige Systemerkrankung des hämatopoetischen Apparates (Hyperplasie des retikuloendothelialen Apparates). *Virchows Arch. f. path. Anat.*, 1924, 252, 519–549. (Cited by Abt and Denenholz [1] and Freud, Grossman, and Dragutsky.[27])
13. Wallgren, A. Systemic reticuloendothelial granuloma (nonlipid reticuloendotheliosis and Schüller-Christian disease). *Am. J. Dis. Child.*, 1940, 60, 471–500.
14. Krahn, H. Reticuloendotheliale Reaktion oder "Reticuloendotheliose" (3. Leukämieform?). *Deutsche Arch. f. klin. Med.*, 1926, 152, 179–201. (Cited by Wallgren.[13])
15. Sherman, I. Observations on reticulo-endothelial cells in septic jaundice. *Arch. Path.*, 1929, 7, 78–83.
16. Uher, V. Ein Beitrag zu den sogenannten Reticuloendotheliosen. *Virchows Arch. f. path. Anat.*, 1933, 289, 504–509.
17. Klostermeyer, W. Über eine sogenannte aleukämische Reticulose mit besonderer Beteiligung des Magen-Darmkanales. *Beitr. z. path. Anat. u. z. allg. Path.*, 1934, 93, 1–10. (Cited by Wallgren.[13])
18. Paige, B. H. A case of reticulosis. (Abstract.) *Am. J. Dis. Child.*, 1935, 49, 266–267.
19. Paige, B. H. Personal communication.
20. Wollstein, M., and McLean, S. Hodgkin's disease, primary in the thymus gland. Report of a case in an infant. *Am. J. Dis. Child.*, 1926, 32, 889–899.
21. Jaffe, H. L., and Lichtenstein, L. Eosinophilic granuloma of bone. *Arch. Path.*, 1944, 37, 99–118.
22. Glanzmann, E. Infektiöse Retikuloendotheliose (Abt-Letterer-Siwe'sche Krankheit) und ihre Beziehungen zum Morbus Schüller-Christian. *Ann. paediat.*, 1940, 155, 1–8.
23. Gross, P., and Jacox, H. W. Eosinophilic granuloma and certain other reticulo-

endothelial hyperplasias of bone. A comparison of clinical, radiologic, and pathologic features. *Am. J. M. Sc.*, 1942, **203**, 673–687.

24. Erber, L. J. Über sogenannte Retikulose mit Fettspeicherung. *Virchows Arch. f. path. Anat.*, 1931, **282**, 621–629. (Cited by Gross and Jacox.[23])
25. Freund, M., and Ripps, M. L. Hand-Schüller-Christian disease: a case in which lymphadenopathy was a predominant feature. *Am. J. Dis. Child.*, 1941, **61**, 759–769.
26. Galeotti Flori, A., and Parenti, G. C. Reticuloendoteliosi iperplasica infettiva ad evoluzione granuloxantomatosa (tipo Hand-Schüller-Christian). *Riv. di clin. pediat.*, 1937, **35**, 193–263. (Cited by Freund and Ripps.[25])
27. Freud, P., Grossman, L., and Dragutsky, D. Acute idiopathic cholesterol granulomatosis. *Am. J. Dis. Child.*, 1941, **62**, 776–792.
28. Sweitzer, S. E., Winer, L. H., and Cumming, H. A. Reticuloendotheliosis. *Arch. Dermat. & Syph.*, 1939, **40**, 192–199.
29. Merritt, K. K., and Paige, B. H. Xanthomatosis (Schüller-Christian syndrome). Report of a case with necropsy. *Am. J. Dis. Child.*, 1933, **46**, 1368–1392.
30. Grady, H. G., and Stewart, H. L. Hand-Schüller-Christian's disease and tuberculosis. *Arch. Path.*, 1934, **18**, 699–709.
31. Lane, C. W., and Smith, M. G. Cutaneous manifestations of chronic (idiopathic) lipoidosis (Hand-Schüller-Christian disease). Report of 4 cases, including autopsy observations. *Arch. Dermat. & Syph.*, 1939, **39**, 617–644.
32. Farber, S. The nature of "solitary or eosinophilic granuloma" of bone. *Am. J. Path.*, 1941, **17**, 625–626.
33. Ritchie, G., and Meyer, O. O. Reticulo-endotheliosis. *Arch. Path.*, 1936, **22**, 729–737.
34. Goldzieher, M. A., and Hornick, O. S. Reticulosis. *Arch. Path.*, 1931, **12**, 773–782.
35. Dameshek, W. Proliferative diseases of the reticulo-endothelial system. II. Aleukemic reticulosis. Report of a case. *Folia haemat.*, 1933, **49**, 64–85.
36. Glatt, M. A. Xanthoma or lipoid granuloma of the temporal bone (Hand-Christian-Schüller syndrome). *Arch. Otolaryng.*, 1946, **43**, 110–121.
37. Lichty, D. E. Lipoids and lipoid diseases. II. Xanthomatosis. (Schüller-Christian's type). *Arch. Int. Med.*, 1934, **53**, 379–390.
38. Rowland, R. S. Xanthomatosis and the reticulo-endothelial system. *Arch. Int. Med.*, 1928, **42**, 611–674.
39. Chester, W. Über Lipoidgranulomatose. *Virchows Arch. f. path. Anat.*, 1930–31, **279**, 561–602.
40. Chester, W., and Kugel, V. H. Lipoid granulomatosis (type, Hand-Schüller-Christian). Report of a case. *Arch. Path.*, 1932, **14**, 595–612.
41. Sosman, M. C. Xanthomatosis (Schüller-Christian's disease; lipoid histiocytosis). *J. A. M. A.*, 1932, **98**, 110–117.
42. Otani, S., and Ehrlich, J. C. Solitary granuloma of bone simulating primary neoplasm. *Am. J. Path.*, 1940, **16**, 479–490.
43. Lichtenstein, L., and Jaffe, H. L. Eosinophilic granuloma of bone, with report of a case. *Am. J. Path.*, 1940, **16**, 595–604.
44. Dundon, C. C., Williams, H. A., and Laipply, T. C. Eosinophilic granuloma of bone. *Radiology*, 1946, **47**, 433–444.

45. Curtis, A. C., and Cawley, E. P. Eosinophilic granuloma of bone with cutaneous manifestations. *Arch. Dermat. & Syph.*, 1947, **55**, 810–818.

46. Weinstein, A., Francis, H. C., and Sprofkin, B. F. Eosinophilic granuloma of bone. Report of a case with multiple lesions of bone and pulmonary infiltration. *Arch. Int. Med.*, 1947, **79**, 176-184.

47. Fraser, J. Skeletal lipoid granulomatosis (Hand-Schüller-Christian's disease). *Brit. J. Surg.*, 1934–35, **22**, 800–824.

48. Thannhauser, S. J. Eosinophilic granuloma of bone synonymous with Schüller-Christian disease, lipid granuloma, essential xanthomatosis of normocholesteremic type and eosinophilic xanthomatous granuloma. *Arch. Int. Med.*, 1947, **80**, 283–285.

49. Thannhauser, S. J. Eosinophilic granuloma of bone. *J. A. M. A.*, 1947, **134**, 1437–1438.

50. Jaffe, H. L., and Lichtenstein, L. Eosinophilic granuloma of bone. *J. A. M. A.*, 1947, **135**, 935–936.

51. Mallory, T. B. Diseases of bone. *New England J. Med.*, 1942, **227**, 955–960.

52. Letterer, E. Allgemeine Pathologie und pathologische Anatomie der Lipoidosen. *Verhandl. d. Gesellsch. f. Verdauungskr.*, 1939, **14**, 12–51. (Cited by Mallory.[51])

[*Illustrations follow*]

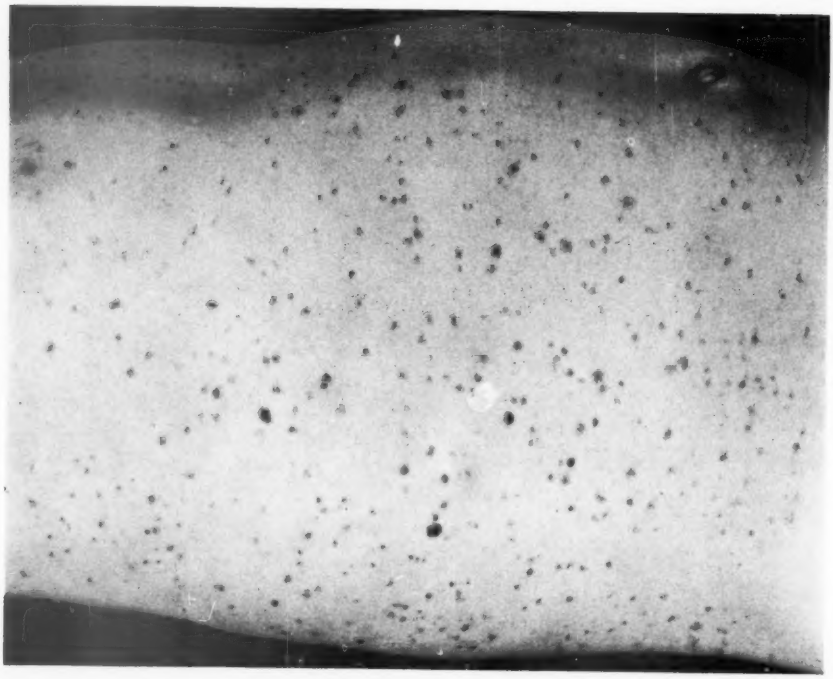
DESCRIPTION OF PLATES

PLATE 7

FIG. 1. Case 1. Lateral view of body showing numerous cutaneous lesions confined chiefly to the torso. Many have hemorrhagic centers.

FIG. 2. Case 1. Roentgenogram of skull showing osseous defects in the right parietal and occipital bones.

1



2

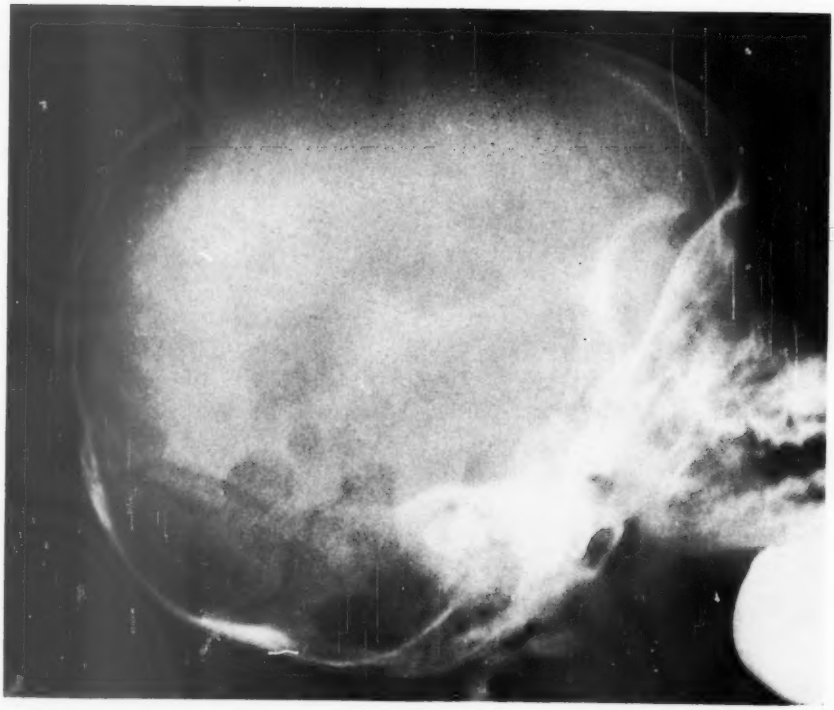
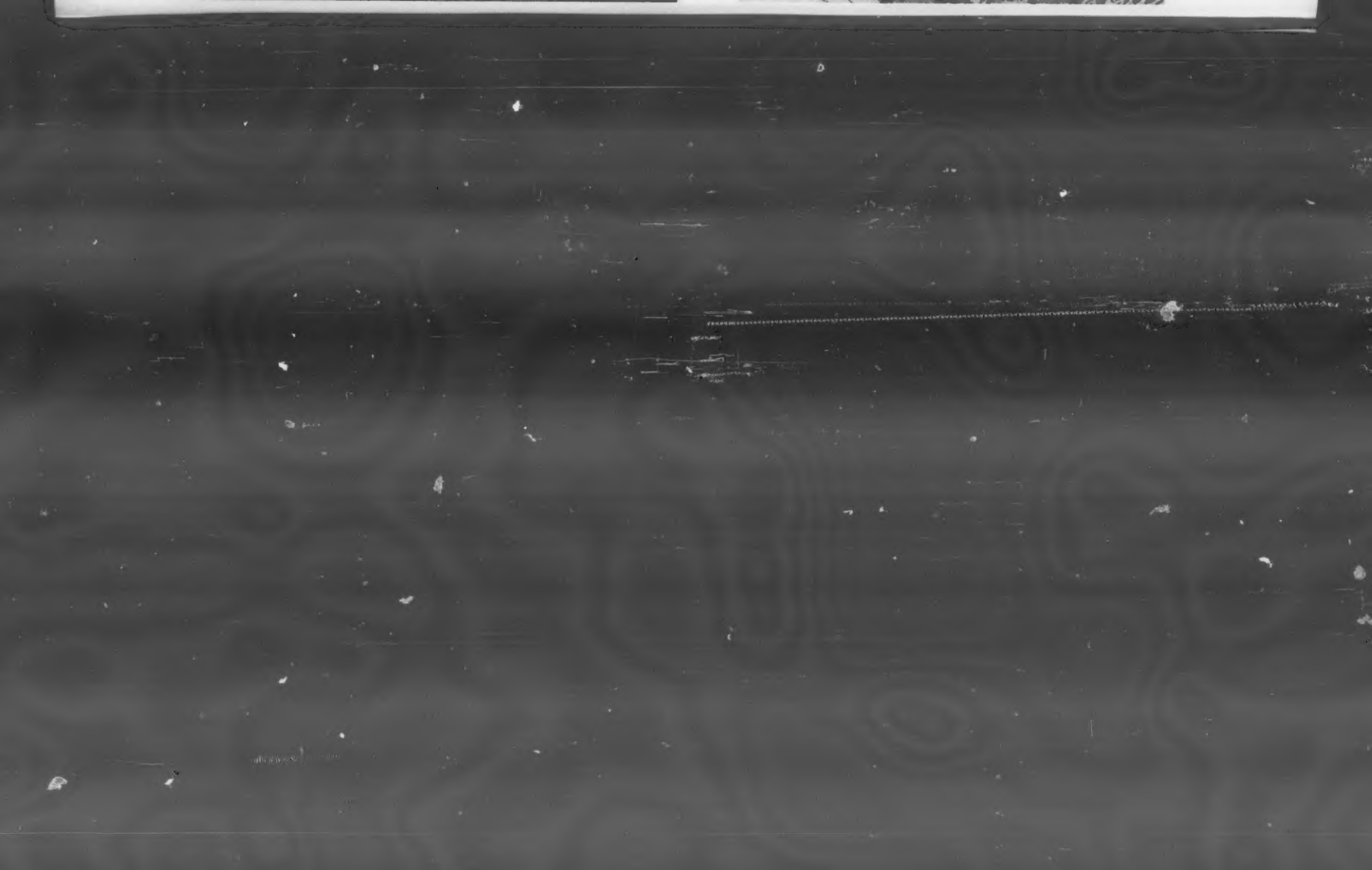
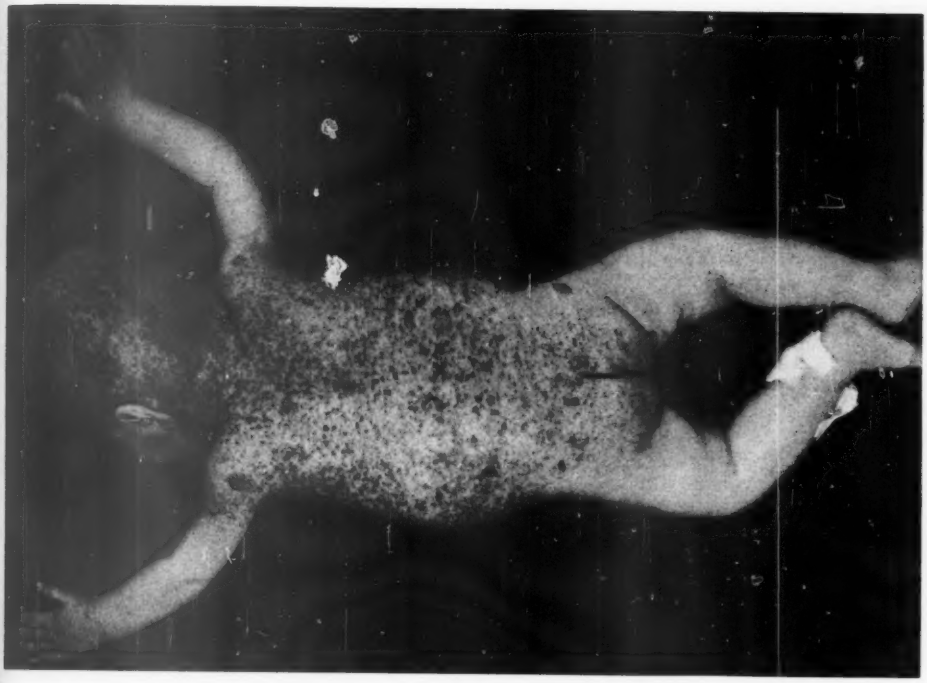


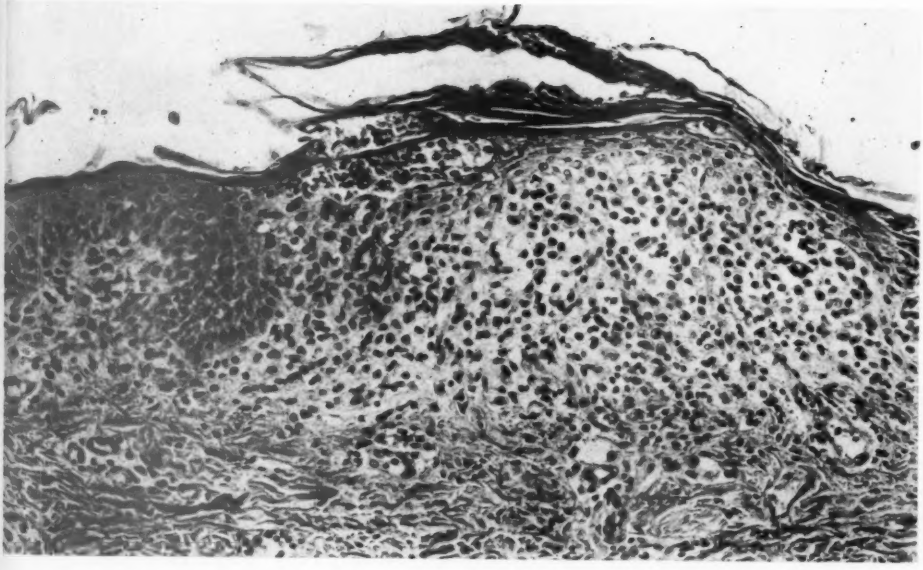
PLATE 8

FIG. 3. Case 2. Dorsal view of body to show diffuse involvement of trunk and scalp with cutaneous lesions. The extremities were spared in this case. The lesions were crusted maculopapules and petechiae. The increased transverse diameter of the body below the level of the ribs is due to hepatic and splenic enlargement.

FIG. 4. Case 1. Cutaneous lesion, showing a collection of atypical mononuclear cells in the corium and atrophy and parakeratosis of the overlying epithelium. There is a minute abscess in the epithelium. For comparison with the photomicrograph of the cutaneous lesion in case 2 (Fig. 5). Hematoxylin and eosin stain. × 186.





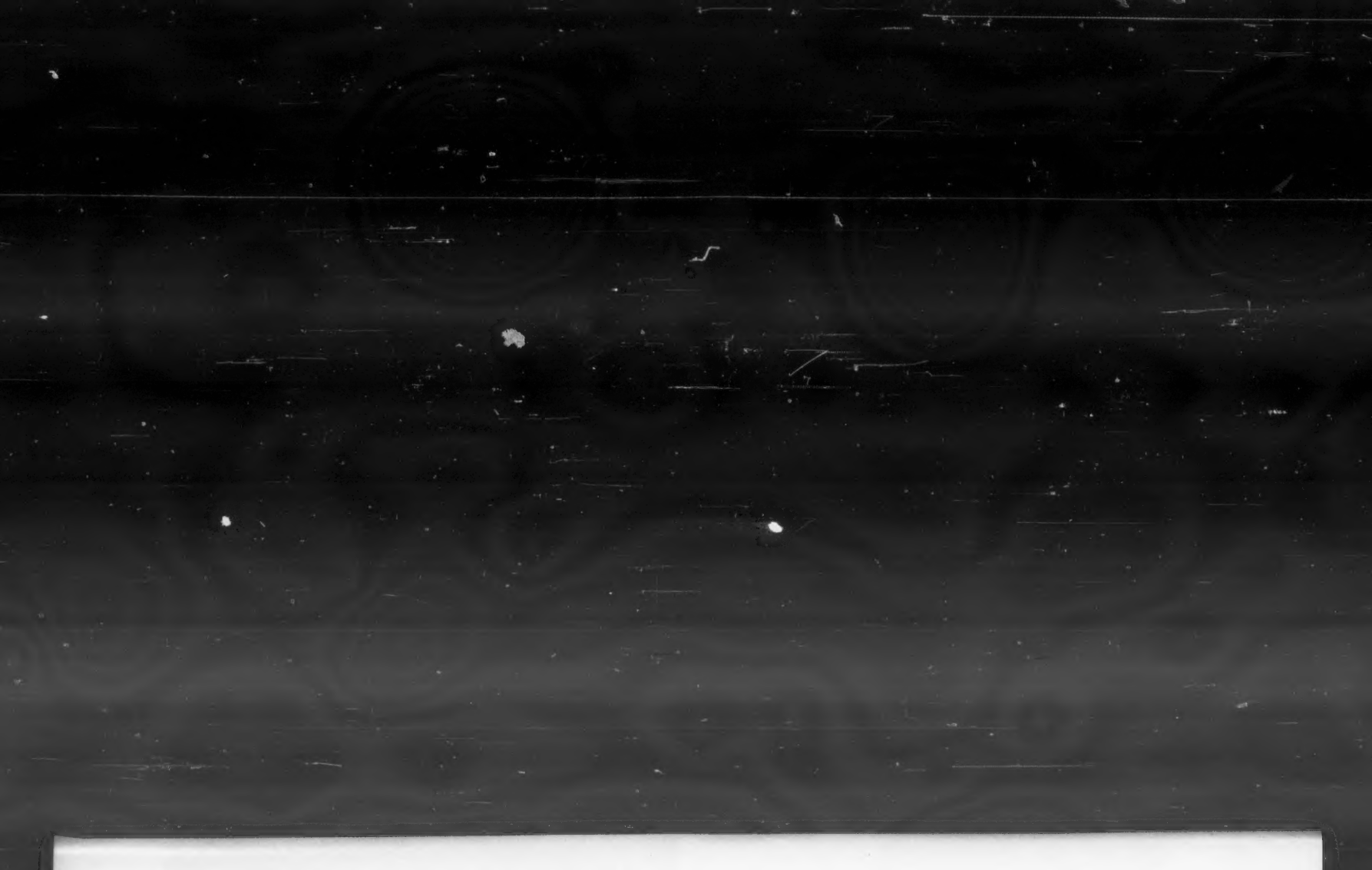


Schafer Nonlipid Reticulo-Endotheliosis

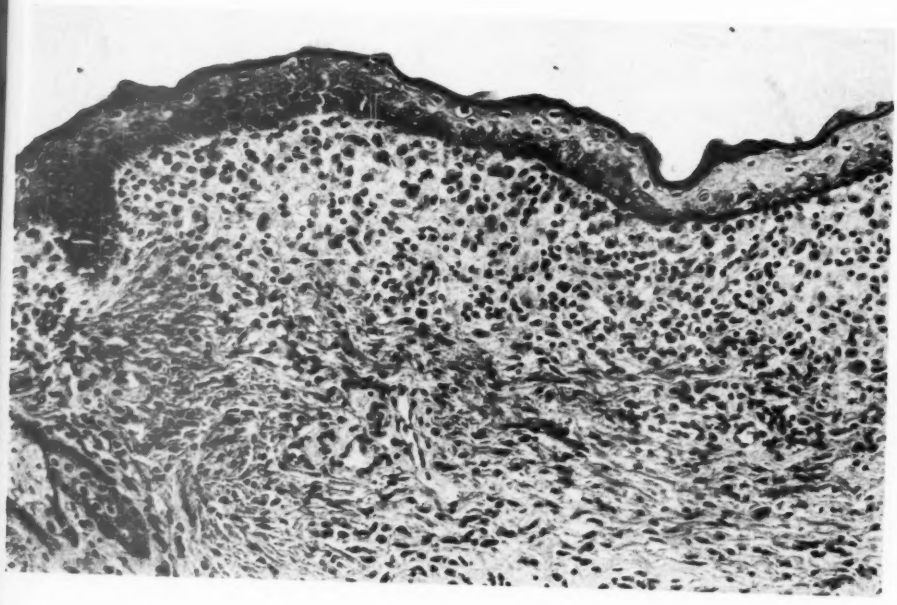
PLATE 9

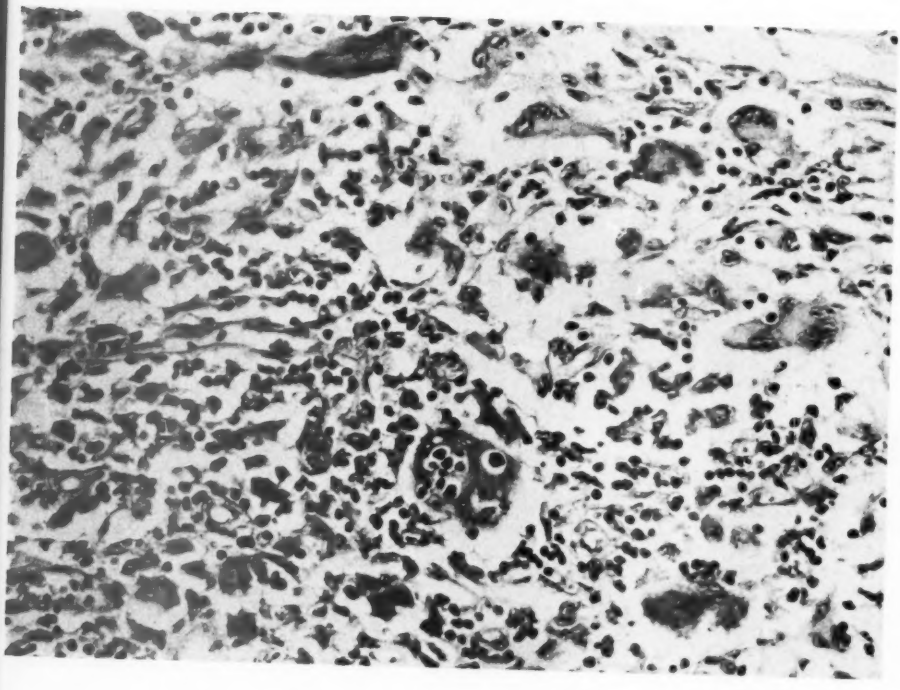
FIG. 5. Case 2. Cutaneous lesion. This photomicrograph shows marked similarity to Figure 4. Hematoxylin and eosin stain. × 186.

FIG. 6. Case 1. Thymus showing numerous giant cells; one in the lower center field shows marked phagocytic activity. Of note are the characteristic mononuclear cells with vesicular nuclei and the irregular outline of the giant cells and mononuclear reticulo-endothelial cells. Hematoxylin and eosin stain. × 342.



S





Schafer

Nonlipid Reticulo-Endotheliosis

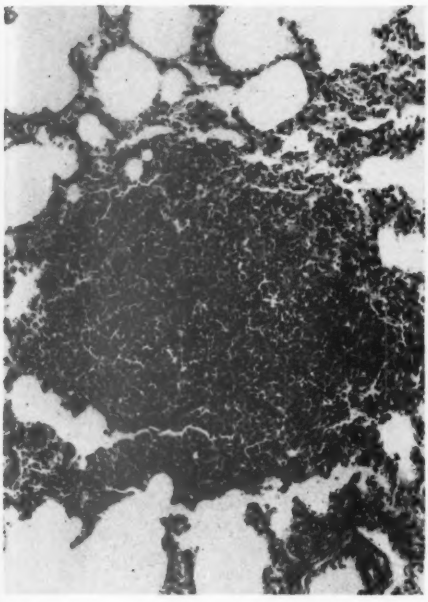
PLATE 10

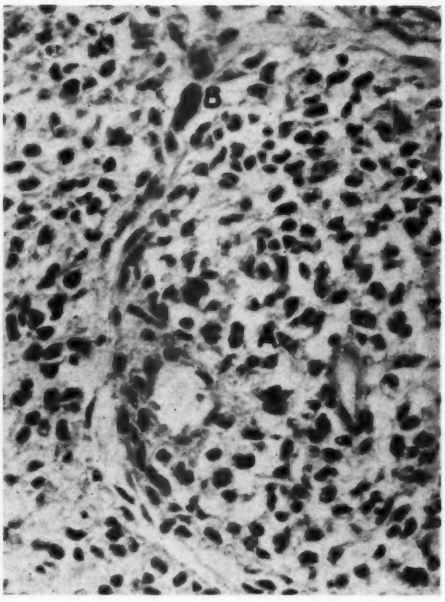
FIG. 7. Case 3. Lung showing a characteristic nodule of reticulo-endothelial cells. Similar cells are present in alveolar septa. Hematoxylin and eosin stain. × 80.

FIG. 8. Case 1. The subepithelial infiltrate in the base of the gingival ulcer. Several mitotic figures are present at A; fibroblastic proliferation and bizarre-shaped nuclear form at B. Hematoxylin and eosin stain. × 342.

FIG. 9. Case 1. Peyer's patch of the ileum completely replaced by large mononuclear cells. The small, dark cells are lymphocytes. Hematoxylin and eosin stain. × 186.

I-49





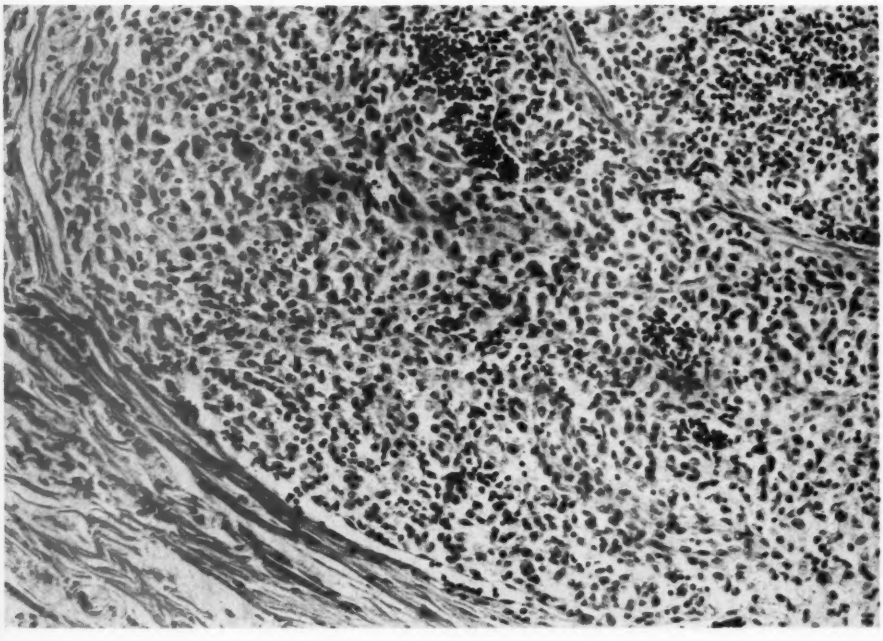
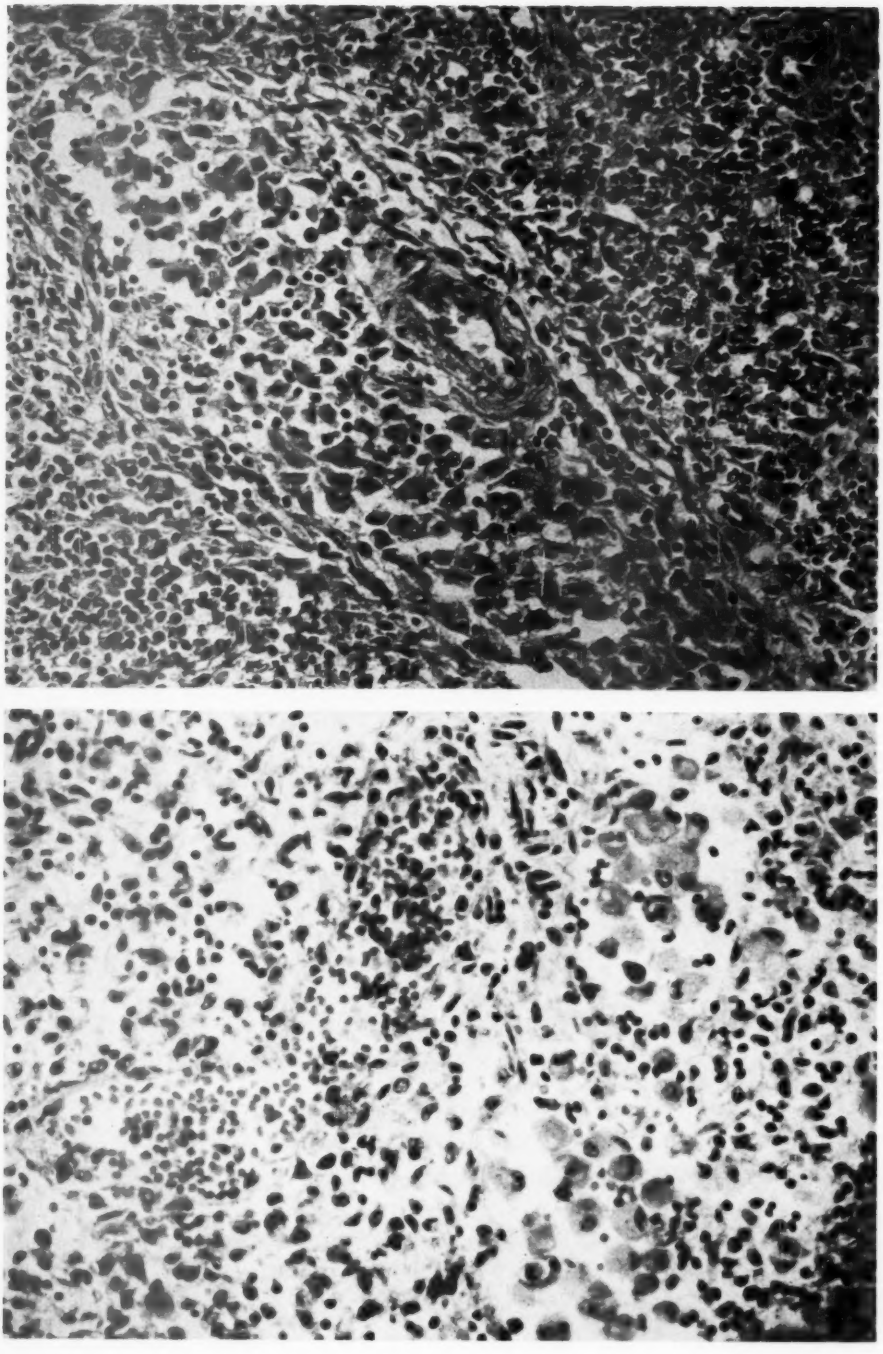


PLATE 11

FIG. 10. Case 1. A nodule of mononuclear cells near a central arteriole in the spleen. There is fibroblastic proliferation surrounding the nodule. A variety of cells may be seen in the splenic pulp. Hematoxylin and eosin stain. × 342.

FIG. 11. Case 2. Lymph node showing collections of large mononuclear cells, some of which have vacuolated cytoplasm, others contain phagocytized cells. Hematoxylin and eosin stain. × 342.



THE NATURE OF THE DOUBLE-CONTOURED AND STRATIFIED INTRACELLULAR BODIES IN SARCOIDOSIS (BOECK-SCHAUMANN)*

GUNNAR TEILUM, M.D.

(From the University Institute of Pathological Anatomy, Copenhagen, Denmark)

The occurrence of double-contoured, stratified, and in some cases calcified, bodies in the pathologically altered lymph nodes and tonsils of patients suffering from sarcoidosis was first observed by Schaumann,[1] who described them in 1917 and found them in particular in tonsils and lymph nodes with a markedly tuberculoid structure. In 1941 Schaumann [2] again described these structures in detail and stated that, as a rule, they are contained in giant cells in which some of them may assume such large size that they occupy the entire giant cell, possibly causing it to burst. In many cases the corpuscles lie in the intercellular spaces in relation to more or less distinct remnants of the giant cells. Schaumann was able to demonstrate the occurrence of such stratified bodies in practically all organs with sarcoidosis, including lungs, spleen, and bone marrow.

Tillgren [3] described them in the granulomata in the pituitary gland in a case of Boeck's sarcoid with diabetes insipidus. The bodies also have been observed by Bergstrand,[4,5] among others, who pointed out that their occurrence supports the diagnosis of Boeck's sarcoid, and by Lindau who, in one of Lemming's cases,[6] described them as concentric, stratified, round, or irregularly ramified bodies which were not calcified or acid-fast and were not stained by Gram's method. In this connection, mention should be made of Weller's observation [15] (1922) of laminated concretions in 3 of 142 cases of "tonsillar tuberculosis, diagnosed histologically." At that time he probably included cases of sarcoidosis.

The intracellular "asteroid bodies" described by Friedman [7] and others in sarcoidosis of the spleen must doubtless be considered as being essentially of the same nature.

PREVIOUS VIEWS OF THE NATURE OF STRATIFIED BODIES IN BOECK'S SARCOIDOSIS

Most of the authors who have previously examined these structures have considered them as being products of degeneration of elastic fibrils [4] impregnated with calcium and iron salts, whereas Schaumann [2] has stated that the possible occurrence of elastic threads in these structures is not of decisive importance as to their genesis. The presence of connective tissue fibrils, hairs, and tubercle bacilli in these bodies also

* Received for publication, January 26, 1948.

is said to have been ascertained. Moreover, Schaumann [2] referred to Metchnikoff's production of quite similar bodies by the inoculation of tubercle bacilli into the Algerian gerbil. In accordance with the marked resistance of this rodent to tuberculosis, the tubercles of the inoculated gerbil as a rule show no signs of necrosis, a fact which might correspond to the findings in Boeck's sarcoid. Considering Metchnikoff's observations and the view of Boeck's sarcoid as being of tuberculous nature, Schaumann [2] advanced the opinion that tubercle bacilli can be transformed into such bodies. He also believed that the bodies may result from the deposition around elastic fibrils, connective tissue fibrils, or a hair, of substances resulting from disintegrated tubercle bacilli in their acid-fast or nonacid-fast form.

Observations

In a recent publication,[8] as the basis of the morphogenesis of the lesions in Boeck's sarcoid and in other conditions, I described an (allergic) *hyperglobulinosis in the reticulo-endothelial system passing on to hyalinosis (or paramyloidosis)*, representing a typical development, by stages, of the sarcoid lesions and presumably the essential change in their structure. In addition it was demonstrated [8,9] that pathogenetically analogous reactions underlie certain morphologic lesions, *inter alia,* lupus erythematosus disseminatus and atypical and experimental amyloidosis. These findings afford an explanation of the nature of the hyperglobulinemia in these states, and in Boeck's sarcoidosis they explain the development by stages of the granulomata and the extragranulomatous changes from reticulosis through (hyperglobulinotic) precipitation to hyalinosis (paramyloidosis) in and around the granulomata.

These findings also illustrate the absence of necrosis, the intensive red staining of the granulomata by Unna's staining method, and the marked accumulation of plasma cells observed in connection with the perigranulomatous precipitates, marked hyalinosis, and paramyloidosis.

I have considered [8] the described elementary reaction in Boeck's sarcoidosis and the other states mentioned, all of which are accompanied at certain stages by hyperglobulinemia as a characteristic symptom, to be *a morphologic immunity reaction,* which shows the importance of plasma cells and of other reticulo-endothelial cells in hyalinosis and atypical amyloidosis in a number of different states. In the case of Boeck's sarcoidosis, such a view is in complete conformity with the general perception of this disorder as a condition with high immunity (positive anergy).

In my case 2 of Boeck's sarcoidosis [8] in a woman, 28 years of age,

with no bacteria in the expectoration and with a negative Mantoux test on repeated examinations, roentgenologic examination and autopsy showed the picture of a bilateral cavernous pulmonary tuberculosis (with no tubercle bacilli either on inoculation into guinea-pigs or on cultivation), combined with typical sarcoidosis in the spleen, lymph glands, liver, and lungs. The pulmonary changes, as well as the fact that the patient's brother had died of tuberculosis, favored tuberculosis as the cause in this case. On the other hand, the highly marked immunity manifested itself through widespread lesions of sarcoidosis (passing on to very marked paramyloidosis) in the reticulo-endothelial system, in particular in the spleen and in the lymph glands, and through the absence of bacteria on inoculation and cultivation from the cavernous foci.

In general, the typical granulomata of Boeck in the spleen and lymph nodes were subject to hyaline or paramyloid transformation, wholly or in part. Many of the granulomata in the spleen, lymph nodes, and lungs were found to contain stratified bodies in accordance with the descriptions given by Schaumann [2] and Skavlem and Ritterhoff.[10] In some instances they were ring-shaped, but usually they were concentrically stratified, double-contoured, almost everywhere situated in giant cells, filling them out wholly or in part (Fig. 1), and often several occurred within the same granuloma (Fig. 2). The bodies stained deep red by Unna's method, most frequently a still deeper red than that of the granulomata and the protoplasm of the plasma cells. This is considered indicative of a content of ribose nucleotide, which is regarded as characteristic of the protein formation in cells and presumably gives rise to their basophilia. In the centers of some of the bodies, there could be demonstrated oblong structures, resembling foreign bodies, surrounded by concentric stratified precipitated material. A conspicuous feature in this case and in other cases of widespread sarcoidosis in the same phase was the occurrence of numerous foreign body giant cells in which the nuclei were placed quite irregularly (Fig. 3) and which resembled osteoclasts. Generally, comparatively few giant cells are seen in this disease. By means of Unna's and Masson's staining methods it was possible in several areas corresponding to the presumably earlier stages of the precipitation to demonstrate intensely red-staining crystalline precipitates or confluent hyaline droplets in direct relation to protoplasm of the reticulum cells and also of a deep red color (Fig. 4). This confirmed the impression that these were products from an intracellular globulin precipitate. Impregnation with iron and calcium may take place later on.

On the basis of these and my previous findings,[8] I believe that it has

been rendered highly probable that the stratified bodies in Boeck's sarcoidosis are due to biochemical (partly crystalline) precipitates of the nature of a globulin in the cytoplasm, where they behave like foreign bodies. The supposition that they are a product of transformed tubercle bacilli seems to me to be improbable. The nature of the change as a *hyperglobulinotic* reaction, expressing an especially high degree of immunity, fully explains the occurrence of stratified bodies in Metchnikoff's inoculation experiments referred to above and in the pathologically changed tissue in Boeck's sarcoidosis. The occurrence of "transformed tubercle bacilli," as supposed by Schaumann,[2] may be considered theoretically for certain organs such as the lungs, whereas the lesions in sarcoidosis with regard to localization (spleen, lymph nodes, posterior lobe of pituitary body) and structure (extragranulomatous changes) appear as a reticulosis which cannot presuppose the occurrence of tubercle bacilli in these organs. (See also Lemming's [11] production of typical lesions of Boeck at the site of injection after the administration of B.C.G. vaccine to a patient with histologically verified Boeck's sarcoidosis and persisting negative Mantoux's reaction.)

The view advanced here does not preclude, however, that an inoculation with tubercle bacilli, as in Metchnikoff's experiment, or the occurrence of tubercle bacilli in certain tissues, *e.g.*, in the lungs, may give rise to the same morphologic immunity reaction.

Concentric stratified globulinoid precipitates which, as far as structure, similarity of color, and a reaction like that to foreign bodies are concerned, bear a close resemblance to the stratified bodies in Boeck's sarcoidosis have been demonstrated also in the kidneys in plasma cell myeloma (Apitz [12]), an essentially different disorder which, however, in common with sarcoidosis displays hyperglobulinosis and hyperglobulinemia as products of plasma (or other reticulo-endothelial) cells with a tendency to paramyloid precipitation.

Lastly, the monstrous forms of foreign body giant cells containing intracellular, clumpy precipitates in cases of chronic Gaucher's disease [13] and the giant cells which can be observed in atypical amyloidosis after immunization [14] are other instances of a giant cell reaction caused by a biochemical intracellular precipitation in reticulum cells.

Summary

On the basis of the view of Boeck's sarcoidosis advanced in my previous publications and interpreting the morphologic lesions in this disorder as representing a serologic hyaline (paramyloid) precipitation, having as its starting point a globulin-precipitate (allergic hyperglobu-

linosis) in the reticulo-endothelial system, the "peculiar corpuscles" described by Schaumann as being present in the tissue of sarcoidosis (Boeck-Schaumann) are considered representative of a hyperglobulinotic (hyaline or crystalline) precipitate in the cytoplasm of the reticulo-endothelial cells.

The staining reactions of the precipitates with Unna's and Masson's methods are identical with those of the pre-hyaline precipitates and of the cytoplasm in plasma cells and the reticulum cells of the granulomata, and must be considered indicative of a content of ribose nucleotide.

In the proper stages of sarcoidosis, the crystalline precipitates give rise to the occurrence of numerous foreign body giant cells.

REFERENCES

1. Schaumann, J. Recherches sur le lupus pernio et ses relations avec les sarcoïdes cutanées et sous-cutanées. *Nord. med. Ark.*, 1916–17, **49**, Pt. 2, No. 17, 81 pp.
2. Schaumann, J. On the nature of certain peculiar corpuscles present in tissue of lymphogranulomatosis benigna. *Acta med. Scandinav.*, 1941, **106**, 239–253.
3. Tillgren, J. Diabetes insipidus as a symptom of Schaumann's disease. *Brit. J. Dermat.*, 1935, **47**, 223–229.
4. Berg, S., and Bergstrand, H. Beitrag zur Klinik und Pathologie der benignen Lymphogranulomatose. *Beitr. z. Klin. d. Tuberk.*, 1937, **90**, 536–556.
5. Bergstrand, H. Discussion of article by A. Lindau. So-called chronic miliary tuberculosis. *Acta path. et microbiol. Scandinav.*, 1936, suppl. 26, p. 168.
6. Lemming, R. An attempt to analyze the tuberculin anergy in Schaumann's disease (Boeck's "sarcoid") and uveoparotid fever by means of BCG vaccination. *Acta med. Scandinav.*, 1940, **103**, 400–429.
7. Friedman, M. Sarcoidosis of the spleen. Report of a case with autopsy and a study of intracellular "asteroid bodies." *Am. J. Path.*, 1944, **20**, 621–635.
8. Teilum, G. Allergic hyperglobulinosis and hyalinosis (paramyloidosis) in the reticulo-endothelial system in Boeck's sarcoid and other conditions. A morphologic immunity reaction. *Am. J. Path.*, 1948, **24**, 389–407.
9. Teilum, G. Hyperglobulinemia, periarterial fibrosis of the spleen, and the wire loop lesion in disseminated lupus erythematosus in relation to allergic pathogenesis. *Am. J. Path.*, 1948, **24**, 409–427.
10. Skavlem, J. H., and Ritterhoff, R. J. Coexistent pulmonary asbestosis and sarcoidosis. *Am. J. Path.*, 1946, **22**, 493–517.
11. Lemming, R. Development of Boeck's sarcoid at the place on the skin where a BCG vaccination had been made in a case of Schaumann's disease. *Acta med. Scandinav.*, 1942, **110**, 151–160.
12. Apitz, K. Die Paraproteinosen. *Virchows Arch. f. path. Anat.*, 1940, **306**, 631–699.
13. Teilum, G. Die Gauchersche Krankheit. *Acta med. Scandinav.*, 1943–44, **116**, 170–190.
14. Doerken, E. Histologische Untersuchungen bei Serumpferden mit besonderer Berücksichtigung der Amyloidablagerungen. *Virchows Arch. f. path. Anat.*, 1932, **286**, 487–525.
15. Weller, C. V. Tonsillar tubercles containing intracellular concretions simulating foreign body pseudotubercles. *Ann. Otol., Rhin. & Laryng.*, 1922, **31**, 110–123.

DESCRIPTION OF PLATE

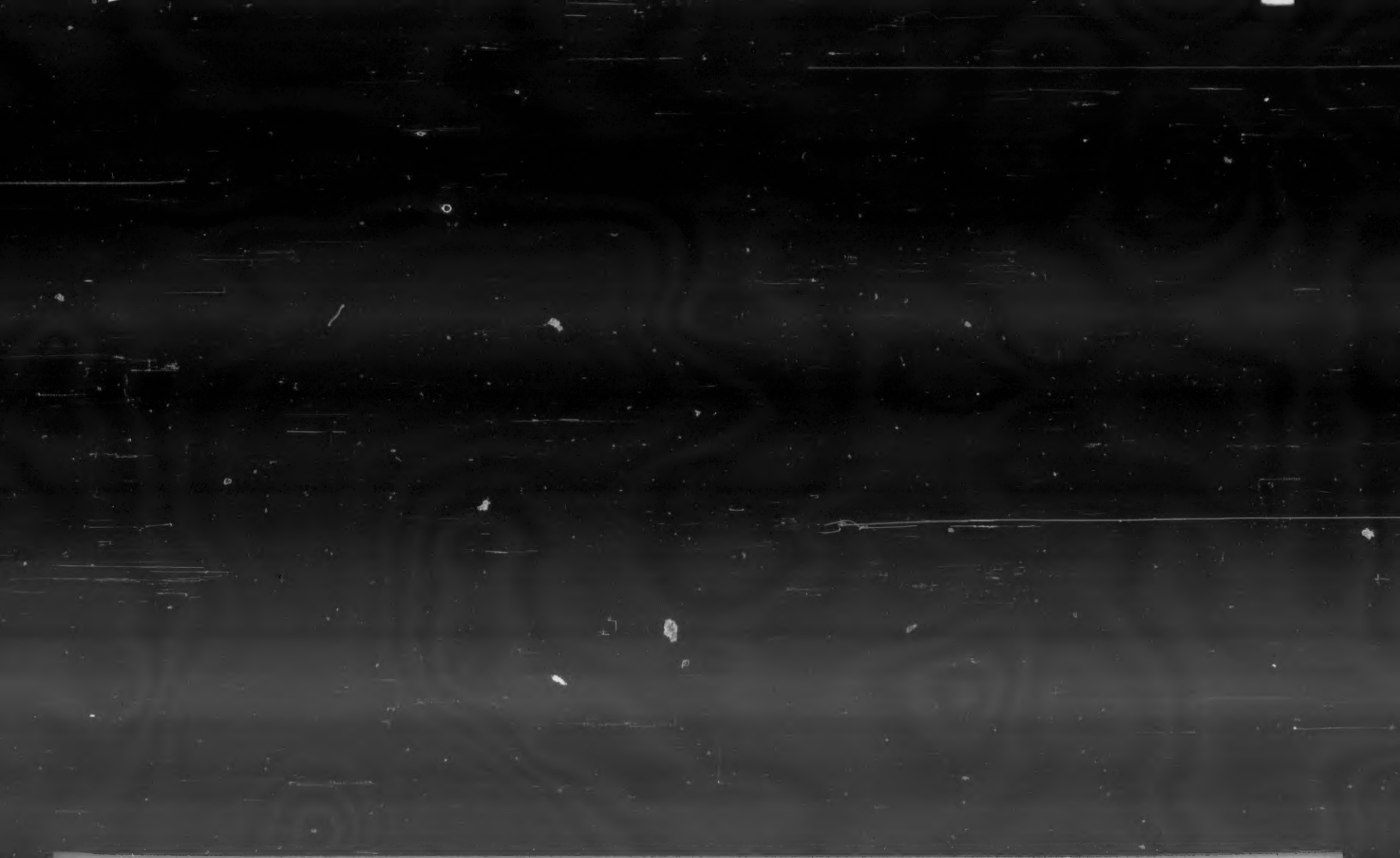
PLATE 12

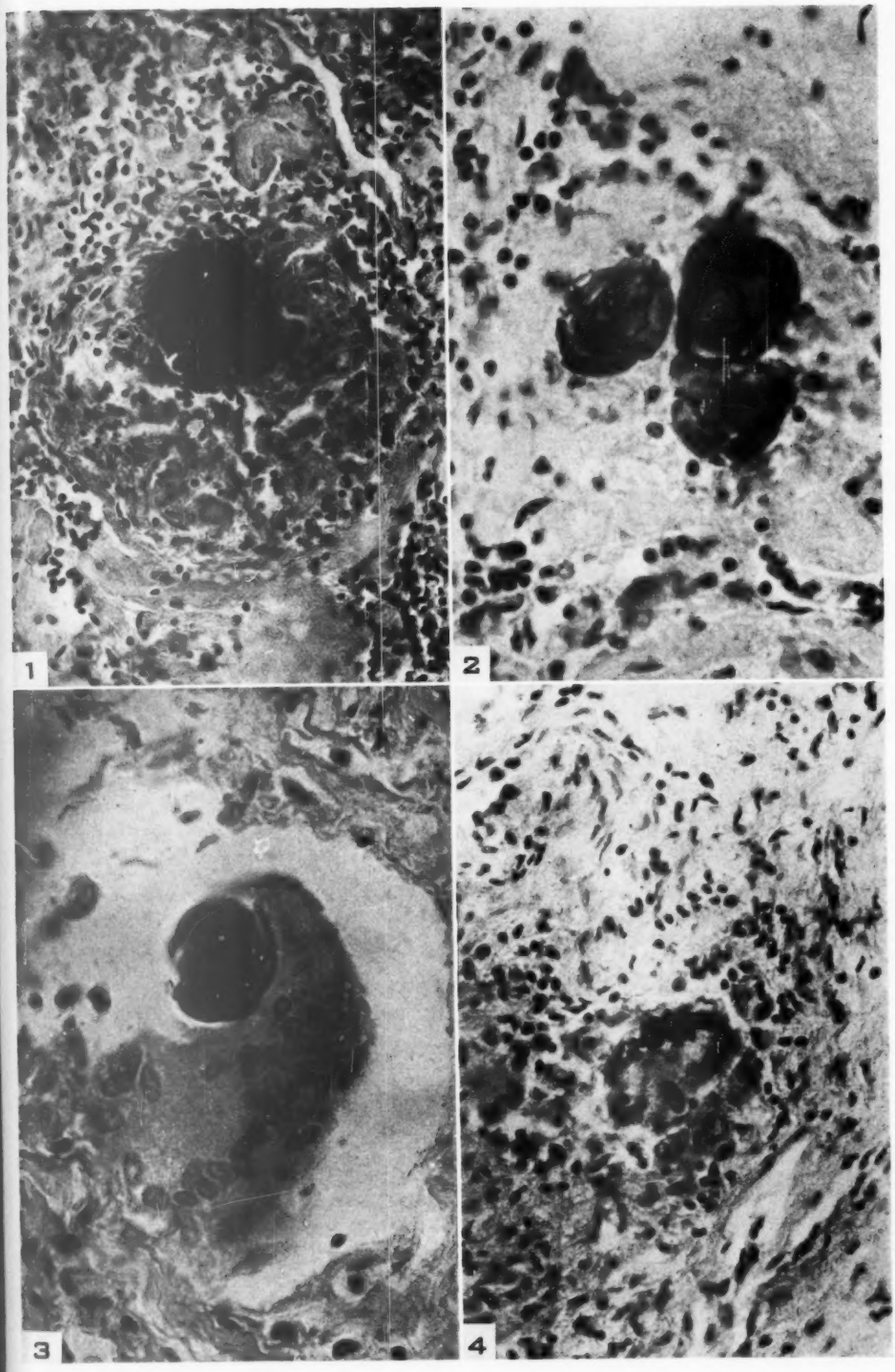
FIG. 1. Stratified body completely filling a giant cell in a granuloma in a lymph node in sarcoidosis. Unna's stain. × 270.

FIG. 2. Three stratified bodies in the same granuloma in sarcoidosis. Unna's stain. × 370.

FIG. 3. Foreign body reaction around a stratified body. Unna's stain. × 370.

FIG. 4. Precipitation of confluent hyaline droplets in direct relation to disintegrated cytoplasm of reticulum cells. Unna's stain. × 370.





Toilum

Intracellular Bodies in Sarcoidosis

THE HISTOGENESIS OF CLEAR CELL PAPILLARY CARCINOMA OF THE SKIN *

Y. Liu, M.D.

(From the Department of Pathology, Peiping Union Medical College, Peiping, China)

Clear cell papillary carcinoma, so designated because of its predominating clear cuboidal-shaped cells arranged in papillary manner, is a variety of basal cell carcinoma. As far as I am aware, this tumor rarely has been discussed in the literature and its origin has not been fully investigated. The so-called simple adenoid carcinoma, believed by Ewing [1] to originate in hair follicles and in which the tumor exhibits papillary or alveolar masses lined by cuboidal or low-cylindric and occasionally squamous cells, seems to be morphologically similar to this tumor. The slowly growing "cubo-celled carcinomata" mentioned by Krompecher,[2] Bloodgood,[3] and Hazen [4] also have clinical and histologic similarities.

Clear cell papillary carcinoma is comparatively rare. Among 700 cases of carcinoma of the skin (including those arising from the penis and vulva) examined in the surgical pathologic laboratory of the Department of Pathology of the Peiping Union Medical College, only 4 such tumors have been seen. The purpose of this report is to present the clinical and histologic features of these 4 tumors and to suggest that they were derived from hair follicles.

Report of Cases

Case 1

A student, 20 years of age, came to the surgical out-patient department of the Peiping Union Medical College Hospital on January 18, 1941, because of a firm, bean-sized nodule in the axillary region. The exact duration of the tumor was not known. It was slowly growing, movable, and cystic. It was excised under local anesthesia. There was no evidence of recurrence 6 months after the operation.

Clinical Diagnosis. Sebaceous cyst or hemangioma.

Pathologic Examination

The specimen consisted of a tumor nodule, well encapsulated, 1.5 cm. in diameter, covered on one side by a piece of skin. Section revealed grayish, homogeneous tissue containing many small cystic spaces filled with blood clot. Microscopically, the tumor was made up of papillary or alveolar masses composed chiefly of clear cuboidal and low-cylindric cells with a varying amount of connective tissue stroma (Fig. 3). The cells near the base of the papillary growth were low-cylindric, showing palisade formation, while the more superficial cells were polyhedral and irregularly arranged and the cytoplasm was mostly clear or scanty.

* Received for publication, December 12, 1947.

The clear cells had prominent borders and rather small pyknotic nuclei. The cylindric cells had finely granular cytoplasm, although they were often quite clear. Mitotic figures were seen only occasionally among the cylindric cells. The most superficial cells (near the skin) were prickle cells showing a tendency to cornification but no definite pearl formation. The papillary masses were closely packed, but occasionally there were crevices or clear spaces. In between them was either thin fibrous or dense hyalinized tissue. Scattered among the clear cells were small, circular, glassy epithelial products (hair shafts) surrounded by flattened or concentrically arranged tumor cells (Fig. 10). These structures stained reddish by Masson's method and yellowish with van Gieson's stain. When Foot's silver method was used they were blackish in varying degrees of intensity, being lighter in the center and deeper at the periphery. With this technic, occasionally a deeply stained, membrane-like object could be seen surrounding the glassy "hair shaft." Similar structures were seen in normal hair follicles with this method. Best's carmine staining of the neoplastic tissue revealed abundant glycogen in the cytoplasm of both clear and nonclear cells.

Diagnosis. Slowly growing, clear cell papillary carcinoma of hair-follicular origin.

Case 2

A woman student, 21 years of age, was seen in Douw Hospital, Peiping, in May, 1938, complaining of a subcutaneous growth on the left arm of about 2 years' duration. It had grown slowly and was symptomless. At the time of her visit the tumor had reached a diameter of about 1.5 cm.

Pathologic Examination

The specimen consisted of a roundish tumor mass measuring 1.5 by 1.0 by 0.8 cm. The cut surface revealed homogeneous whitish tissue with irregular blood-stained spaces. Microscopically, the tumor presented a picture identical with that of case 1 (Fig. 4).

Diagnosis. Slowly growing, clear cell papillary carcinoma arising from a hair follicle.

Case 3

A housewife, 48 years of age, was admitted to the William Porter Hospital, Techow, Shantung, on July 10, 1940, because of a slowly growing tumor of the right thigh of 20 years' duration. When first noticed, the tumor was about the size of a bean, movable beneath the skin. Three years before admission, it had been incised by a country doctor and grew rapidly thereafter. At the time of hospitalization it was about 12 cm. in diameter.

Pathologic Examination

The specimen consisted of a rectangular block of tissue measuring 2.0 by 1.6 by 0.8 cm. One edge was covered by skin. The cut surfaces showed a grayish tissue containing cystic spaces (Fig. 2). Micro-

scopically, the picture was much the same as that seen in the previous cases (Fig. 5). Scattered among the clear cells were occasional, large, foamy cells containing fat globules, as shown when stained by sudan III. In many places the basal cells showed squamous changes and were heavily loaded with glycogen granules.

Diagnosis. Slowly growing, clear cell papillary carcinoma arising from a hair follicle.

Case 4

A farmer, 48 years of age, was seen in the William Porter Hospital, Techow, Shantung, in November, 1940, because of a small mass of about 2 years' duration on his right upper eyelid. At first the tumor had grown slowly, but had assumed a more rapid course following needling and the use of a Chinese plaster, 6 months after its appearance. For the past few months the tumor had bled frequently and there was a continuous discharge of pus and yellowish fluid.

On admission, the general physical and laboratory findings were not remarkable. Locally, an ulcerated tumor the size of a hen's egg was seen to rest upon the arcus infra-orbitalis. It was hard, immobile, and bled easily when touched. The right pre-auricular region showed a small, freely movable lymph node. The tumor was excised and the eyeball enucleated.

Pathologic Examination

The specimen consisted of a tumor measuring 3.5 by 3.0 by 5.0 cm., an eyeball, its attached muscles, and a small lymph node 0.8 cm. in diameter. The covering skin was adherent and showed ulceration and necrosis at its lower margin. Section revealed grayish neoplastic tissue interlaced by thin, glistening stroma (Fig. 1). The eyeball and the lymph node were free from tumor. Microscopically, the tumor showed clear cells, polyhedral prickle cells, and low-cylindric basal cells. The basal cells were actively growing, assuming a spindle shape in places. Mitotic figures were seen occasionally among the palisades of basal cells. The characteristic epithelial products seen in the previous cases also were observed.

Diagnosis. Slowly growing, clear cell papillary carcinoma of hair-follicular origin.

Clinical and Pathologic Considerations

The clinical features of the 4 cases are listed in Table I. All of the tumors grew slowly at the start. A history of rapid growth following improper surgical intervention was noted twice. The duration of the tumor varied from 2 to 20 years. All were located subcutaneously and were distributed in hair-bearing areas. The size of the tumors ranged from 1 to 12 cm. in diameter. A palpable lymph node was present in one instance but metastasis was not found.

All tumors were situated just beneath the epidermis. In 2 instances they protruded above the skin surface and exhibited an ulcerative,

nodular growth with purulent discharge. Cut section showed grayish neoplastic tissue forming papillary nodules or alveolar spaces. In areas there were irregular, yellowish, necrotic spots mingled with small cystic spaces filled with blood clot.

The histologic picture described above reveals several points of special interest.

Cytologic Features. Neoplastic cells were of three distinct types: clear, basal, and prickle. The cells constituting the major part of the tumor were polyhedral or cuboidal. They had distinct cell borders and small, eccentrically located, pyknotic nuclei. Some contained finely granular cytoplasm with clear vacuoles. Morphologically, these cells

TABLE I

Summary of Clinical Data of 4 Cases of Clear Cell Papillary Carcinoma of Skin

Case no.	Age	Sex	Duration of growth	Location	Size	Ulceration	Metastasis
1	20	M	Unknown	Subcutaneous tissue of left axilla	3 x 2 x 1 cm.	−	−
2	21	F	2 years	Subcutaneous tissue of left arm	8 x 1 x 1.5 cm.	−	−
3	48	F	20 years	Subcutaneous tissue of left thigh	12 x 10 x 10 cm.	+	−
4	48	M	2 years	Right upper eyelid	3 x 3.5 x 3.5 cm.	+	−

were very much like those which surround the internal root sheath cells above the neck of the hair papillae. In the neoplastic tissue there was also a palisade arrangement of basal cells closely associated with the clear cells. These cells correspond to the basal cells in palisade arrangement above the neck of the normal hair papillae. The prickle cells were found near the top of the tumor, which means that the superficial part of the tumor was better differentiated, a condition often seen also in the squamous cell carcinoma or nevus. Maximow and Bloom,[5] Mallory,[6] and Haythorn,[7] in their study of basal cell tumors of hair follicles, claimed that the basal cells are in reality true prickle cells.

Papillary Arrangement. All of the tumors showed papillary or alveolar growth and the papillae were closely arranged, leaving occasional crevices between them (Fig. 6). The fibrous stalk varied in amount and character and was made either of irregular bands of collagenous or hyalinized tissue or of acutely proliferating young fibrous tissue containing many capillaries. The significance of the papillary growth is self-evident, as the tumor is slowly growing and of a low degree of malignancy. The picture may become one of compact masses of neoplastic tissue, as shown in case 4.

Epithelial Products. In all instances, there were small cylindric formations which seemed to represent aborted or immature hair shafts. They could be distinguished from cornified pearls by their structure and from hyalinized substances by special staining methods (Fig. 10).

Glycogen. The glycogen content of the tumors was abundant, as shown by Best's carmine stain (Fig. 8). Lubarsch,[8] in his study of this subject, revealed that actively growing tumors of the skin contain more glycogen than slowly growing tumors, but that tumors arising from skin appendages such as hair follicles or sebaceous glands show the most glycogen in the cytoplasm of the tumor cells. Normal squamous epithelium contains small amounts of glycogen while epithelial cells of the mucous membranes, particularly the vaginal epithelium, show an abundant glycogen content.

Epithelial Fibrils. Under high magnification, intercellular bridges and fibrils were noted easily among the basal cells (Fig. 11). In comparing them with those of the normal hair matrix, no morphologic difference was found. The hair shaft is, in fact, composed of flattened or elongated epithelial cells with intercellular bridges running along the long axis of the matrix cells (Fig. 7).

Discussion

The origin of the so-called Krompecher's basal cell carcinoma [2] has long been a subject of controversy. Krompecher's original thesis was favored by Montgomery.[9] Mallory [10] and Haythorn [7] believed that all basal cell carcinomas of the skin are hair matrix tumors. Stout [11] and Boyd,[12] on the other hand, suggested that this tumor has multiple origins from the skin, hair follicles, and embryonic cell rests. In a recent study, Foot [13] expressed the opinion that the basal cell carcinomas of the skin originate in a distorted primordia of dermal adnexia. Embryonically, basal cells are mother cells of epidermis as well as of skin appendages. Therefore, these cells have multiple potentialities of development. In 1940, Traenkle [14] studied 63 examples of various epithelial neoplasms of the skin, in which 13 presenting pictures identical with Krompecher's basal cell carcinoma were found to show abortive attempts at hair follicle formation. This probably indicates that basal cell carcinomas have various morphologic differences and that the basis for such changes depends upon their types of differentiation.

The clinical features of clear cell papillary carcinoma are those of slowly growing tumors of low malignancy. The histologic pictures are unique. The papillary arrangement of clear cells, the palisade pattern of basal cells, the attempt at hair-shaft formation, and the close re-

semblance of the clear cells to the internal root sheath are evidence in favor of their origin from hair follicles. Compared with the usual hair follicle carcinoma or Mallory's hair matrix carcinoma, these tumors are histologically better differentiated and clinically more benign, although all belong to the same group.

That these tumors are not of sweat gland origin is self-evident. Their structure is quite different from that of sebaceous gland tumors. Hazen [4] and Eller [15] have stated that apocrine gland tumors show papillary cystic structure or diffuse anaplastic growth. Histologically, a well formed sebaceous tumor presents characteristic lobules [16,17] formed by squamous cells with central fatty change. Although in one of my cases there were foamy cells scattered here and there, one can hardly consider them as proper constituents of the tumor, particularly in the presence of infection and ulceration.

Summary and Conclusion

Four cases of cutaneous carcinoma were characterized clinically by slow growth, subcutaneous location, and distribution on the hair-bearing areas of the body, and microscopically by papillary growth and palisade arrangements of low cylindric basal cells and rather disorderly arranged, glycogen-rich, clear cells. The close resemblance of the clear cells and the palisaded basal cells to the outermost layer of the internal root sheath cells, and the finding of immature hair shafts, suggest strongly that they originate in hair follicles.

REFERENCES

1. Ewing, J. Neoplastic Diseases. W. B. Saunders Co., Philadelphia & London, 1940, ed. 4, 907–908.
2. Krompecher, E. Der Basalzellenkrebs. G. Fischer, Jena, 1903. (Cited by Ewing.[1])
3. Bloodgood, J. C. Anesthetics, fractures, dislocations, amputations, surgery of the extremities, and orthopedics. *Prog. med.*, 1904, **4**, 156–158.
4. Hazen, H. H. Skin Cancer. C. V. Mosby Co., St. Louis, 1916, pp. 81–82; 92–101.
5. Maximow, A. A., and Bloom, W. A Textbook of Histology. W. B. Saunders Co., Philadelphia & London, 1935, ed. 2, pp. 329–335.
6. Mallory, F. B. Principles of Pathologic Histology. W. B. Saunders Co., Philadelphia, 1914, pp. 371–373.
7. Haythorn, S. R. Studies on the histogenesis of the so-called "basal-cell carcinoma." *Am. J. Cancer*, 1931, **15**, 1969–2000.
8. Lubarsch, O. Über die Bedeutung der pathologischen Glykogenablagerungen. *Virchows Arch. f. path. Anat.*, 1906, **183**, 188–228.
9. Montgomery, H. Histogenesis of basal-cell epithelioma. *Radiology*, 1935, **25**, 8–23.

10. Mallory, F. B. Recent progress in the microscopic anatomy and differentiation of cancer. *J. A. M. A.*, 1910, **55**, 1513–1516.

11. Stout, A. P. Human Cancer. Lea & Febiger, Philadelphia, 1932, pp. 569–571.

12. Boyd, W. A Textbook of Pathology. Lea & Febiger, Philadelphia, 1934, ed. 2, p. 327.

13. Foot, N. C. Adnexal carcinoma of the skin. *Am. J. Path.*, 1947, **23**, 1–27.

14. Traenkle, H. L. Epithelioma adenoides cysticum tricho-epithelioma, and basal cell cancer. *Arch. Dermat. & Syph.*, 1940, **42**, 822–839.

15. Eller, J. J. Tumors of the Skin. Lea & Febiger, Philadelphia, 1939, pp. 289–290.

16. Dupont, A. Epithéliomas sébacés multiples à point de départ épidermique. Epithéliomas sébacés multiples. *Bull. Soc. franç. de dermat. et syph.*, 1938, **45**, 704–709.

17. Lucien, M., and Créhange, J. L. Epithélioma sébacé du lobule de l'oreille. *Bull. Soc. franç. de dermat. et syph.*, 1939, **46**, 260–263.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 13

FIG. 1. Case 4. Cut surface of a clear cell carcinoma of the skin, showing the papillary and alveolar arrangement of the neoplastic tissue. Natural size.

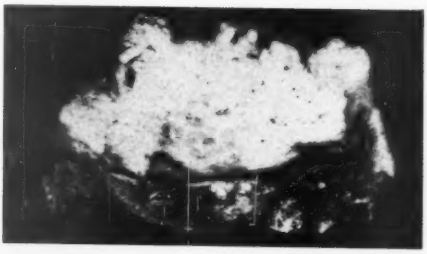
FIG. 2. Case 3. General topography of the tumor, showing its subcutaneous location, alveolar arrangement, invasion into the epidermis, and formation of many large and small cysts. Some of the rete pegs of the epidermis are greatly increased in length. Hematoxylin and eosin stain. × 7.

FIG. 3. Case 1. The characteristic palisade arrangement of low-cylindric cells around the vascular stroma is shown. Hematoxylin and eosin stain. × 155.

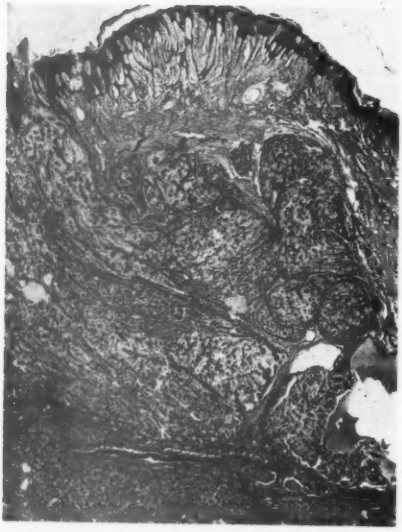
FIG. 4. Case 2. The clear cells of the tumor present an alveolar arrangement. Hematoxylin and eosin stain. × 155.

FIG. 5. Case 3. The palisade arrangement of the tumor cells may be seen. Hematoxylin and eosin stain. × 155.

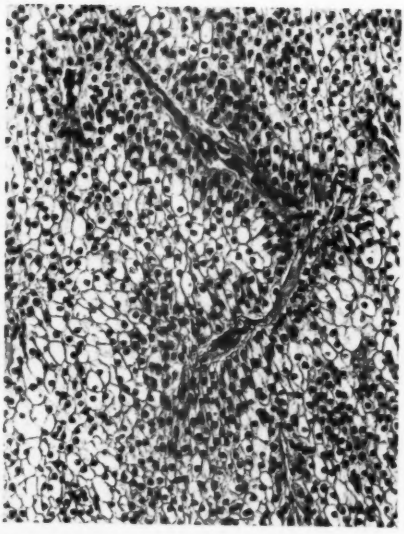
1



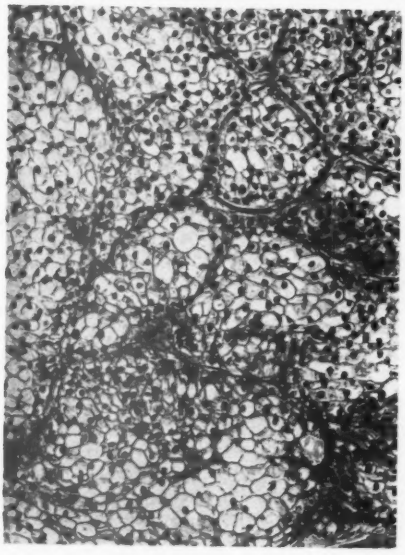
2



3



4



5

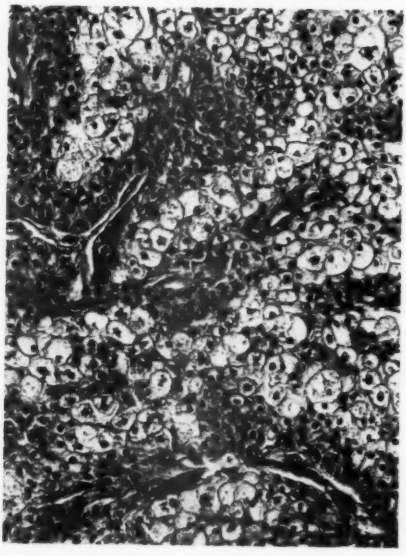


Plate 14

Fig. 6. Case 1. A pseudocyst lined by two layers of flattened epithelial cells. Papillary growth of tumor in lumen. Hematoxylin and eosin stain. × 155.

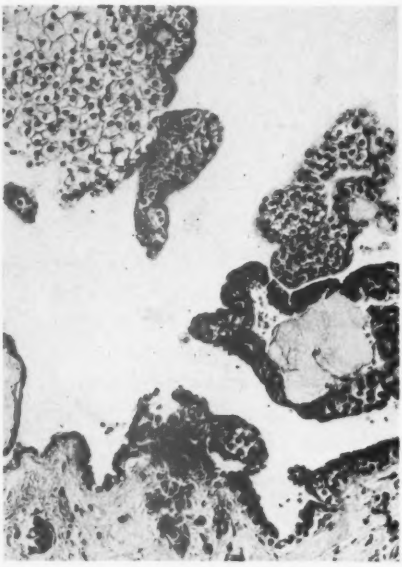
Fig. 7. Epithelial fibrils in normal hair matrix cells. Foot's silver method. × 1250.

Fig. 8. Case 3. Glycogen in tumor cells. Best's carmine stain. × 155.

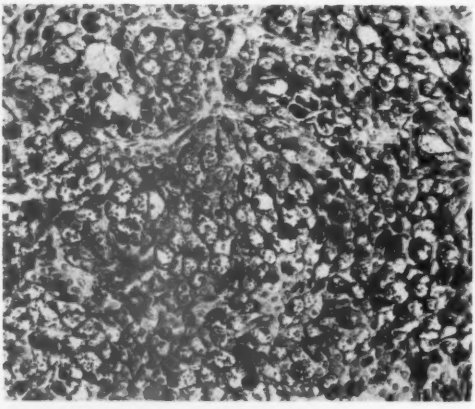
Fig. 9. Immature hair shaft in normal skin. Hematoxylin and eosin stain. × 570.

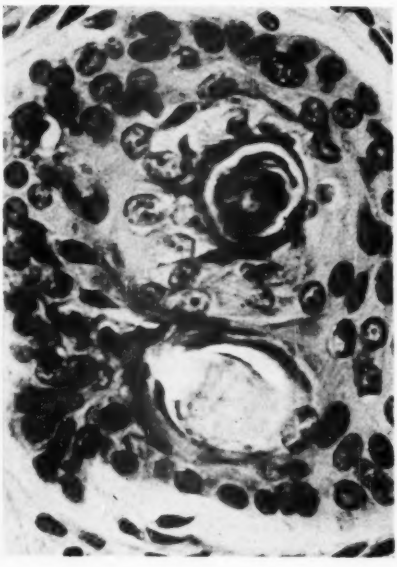
Fig. 10. Case 1. Immature hair shaft in tumor. Hematoxylin and eosin stain. × 570.

Fig. 11. Epithelial fibrils in tumor cells. Foot's silver stain. × 1290.

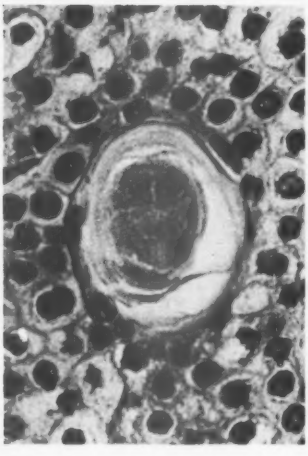


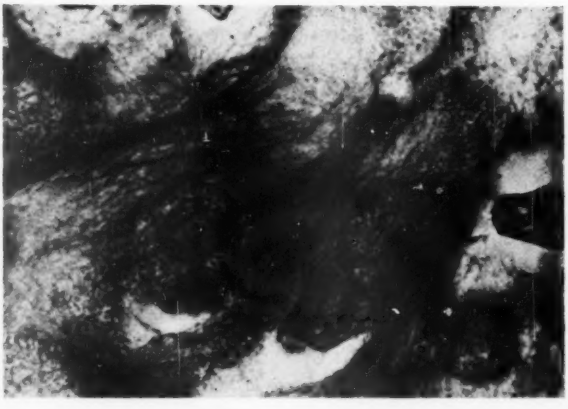
7





9





11

PRIMARY AMYLOIDOSIS, WITH REPORT OF SIX CASES *

DAVID C. DAHLIN, M.D.

(From the Section on Surgical Pathology, Mayo Clinic, Rochester, Minn.)

Primary systemic amyloidosis is an unusual disease, there being only slightly more than 50 cases reported in the literature. It differs from the more common secondary amyloidosis in several respects: (1) Absence of the generally considered specific etiologic factors such as tuberculosis or chronic suppuration, (2) often minimal involvement of the liver, spleen, kidneys, and adrenal glands, which are ordinarily the sites of maximal deposition in secondary amyloidosis, (3) usually considerable deposition in the heart, lungs, skin, striated muscles, and other tissues not often involved in the secondary type, (4) often atypical reactions to the specific amyloid stains, and (5) the occasional occurrence of amyloid tumors.

Wild,[1] in 1886, is credited with reporting the first case of primary amyloidosis. Lubarsch [2] reported 3 cases in 1929, and suggested criteria for the diagnosis. In 1939 Koletsky and Stecher [3] reviewed the literature, tabulated data on 23 cases, and reported a case of their own. Lindsay and Knorp,[4] in 1945, similarly tabulated data on 16 additional examples and reported a case. In 1946 Lindsay [5] reported another case of primary amyloidosis and included those of Brown and Selzer,[6] Golden,[7] Bannick, Berkman, and Beaver [8] and a second case of Pick's [9] in an analysis of the total of 45 cases. He stressed the importance of the cardiac lesions in this disease and pointed out that 23 patients showed signs of cardiac failure during their illness and that, in 18 of these, cardiac failure was considered the immediate cause of death.

In reviewing the literature, it was found that additional examples of this disease have been reported by Barnard, Smith, and Woodhouse [10] (2 cases), Kernohan and Woltman,[11] Bürümcekci,[12] Fowler,[13] Soisalo and Ritama,[14] Ferris,[15] Bell,[16] Eisen [17] (2 cases) and Ranstrom.[18] This makes a total of 57 cases of primary systemic amyloidosis found in the literature. There are, perhaps, others that have been overlooked. It is noteworthy that differences in terminology make for difficulty in locating case reports. Atypical amyloidosis,[9] paramyloidosis,[12] unusual amyloid deposits,[2] idiopathic amyloid disease,[18] and amyloid neuritis [11] are some of the titles under which reports of cases of primary systemic amyloidosis have been found.

Of all the criteria given for the diagnosis of primary systemic amy-

* Abridgment of a thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Pathology.

Received for publication, February 19, 1948.

loidosis, absence of pre-existing or etiologic disease is the only constant clinical and pathologic finding. The staining reactions of the primary amyloid as well as its distribution overlap those seen in secondary amyloidosis. Because of these facts, opinions differ regarding what constitutes an example of primary systemic amyloidosis. Some writers have believed that the cases of Bell [16] and Fowler [13] should not be included.

Of the 57 cases of primary systemic amyloidosis in the literature, information regarding sex and age was available in 55. The average age was 55 years, the oldest patient being 80 and the youngest, 15 years. No significant sex predilection was noted, 30 of the patients were males and 25, females.

More or less complete necropsy reports were available in 54 cases. Cardiac amyloid infiltration was specifically mentioned in 46 instances, and in 25 of these, myocardial insufficiency was considered the cause of death. Involvement of the gastro-intestinal tract was mentioned in 33 cases. In 2 cases [2,19] a clinical diagnosis of carcinoma of the stomach was made. In a third,[7] gastric resection was performed, and it was discovered that diffuse amyloid infiltration had caused pyloric obstruction and hematemesis. Many of the patients with amyloid in the gastro-intestinal tract presented no definite symptoms referable to this phase of the disease, but 5 of them had serious hemorrhage from the gastro-intestinal tract.[2,7,19-21] Amyloid was found in the tongue of 26 patients, in most of whom macroglossia was noted clinically. Pulmonary involvement was noted in 24 cases, in one of which [12] severe amyloid infiltration of the lungs resulted in an extensive shadow in the roentgenogram and was associated with right ventricular hypertrophy. Infiltration of amyloid in striated muscle, which was observed in at least 19 instances, has been blamed for weakness, fatigue, and limitation of motion.[4] Amyloid deposits were present in the skin of about one-fourth of the cases, an important diagnostic feature. It is worthy of note that involvement of the liver and kidneys was found in 21 cases and splenic involvement in 20. In one instance,[8] massive amyloidosis of the liver simulated hepatic cirrhosis clinically. Involvement of bones and joints has been reported.[4,22] Involvement of small arteries and veins of many organs was seen commonly. In one of these, obstruction of vasa nervorum by amyloid in their walls resulted in clinical signs due to ischemic neuropathy.[11] In the case reported by Götze and Krücke,[23] amyloid was present in the peripheral nerves and in the vessels of the brain. This case was probably an example of primary amyloidosis in spite of the associated suppurative renal disease. Neurologic signs due to amyloid occurred in the case described by De Navasquez and Treble.[24] In this brief re-

view, many of the less commonly involved sites have not been mentioned. The intravenous Congo red test may give positive [22] or negative [7] results in cases in which extensive involvement occurs.

Of the 44 cases of amyloid disease in which necropsy was performed at the Mayo Clinic during the 25-year period 1922 through 1946, 7 were examples of primary systemic amyloidosis.* In an eighth case, necropsy was done in 1947. Six of these patients were men and 2 were women. Their ages varied from 40 to 75 years, the average being 56 years. The interval between the onset of symptoms and death varied from 8 months to 4 years. Two of these cases have been reported previously.[8,11] A review of the 6 cases not previously reported follows:

Report of Cases

Case 1

A white woman, 75 years old, who registered at the Clinic on April 21, 1938, gave a history of ankle edema of 2 years' duration. She had lost 30 lb. (13.6 kg.) in the 10 months preceding registration and had vomited occasionally during the last 2 months. The blood pressure was 140/80 mm. of Hg. The liver was enlarged and firm. Albuminuria was graded 2 (on the basis of 1 to 4, in which 1 represents the lowest concentration of albumin). The hemoglobin was 12 gm. per 100 cc.; erythrocytes, 4,860,000; leukocytes, 10,200 per cmm. A flocculation test for syphilis gave negative results. A roentgenogram of the chest showed enlargement of the heart and congestion in the bases of both lungs.

During the next 2 months dyspnea on exertion, orthopnea, and nocturnal dyspnea developed. Large, discrete cervical nodes were noted in June, 1938, when there was moderate pitting edema of the lower extremities. An electrocardiogram showed a rate of 98 per minute, slurred QRS_1 and QRS_3, notched QRS_2, isoelectric T_2, inverted T_3, and low amplitude QRS in all leads. The patient failed rapidly and died on June 29, 1938.

At necropsy the peritoneal cavity contained about 300 cc. of clear fluid and each pleural cavity about 2 l. of similar fluid. The heart weighed 468 gm., and the right auricle and right ventricle were moderately dilated. The spleen weighed 144 gm.; the liver, 1850 gm.; and the kidneys, 255 gm. The cervical, mediastinal, and abdominal lymph nodes were firm, discrete, and measured up to 4.0 cm. in diameter. Their cut surfaces were glistening and somewhat translucent.

On microscopic study the walls of the small arteries and arterioles of all tissues examined were found to be infiltrated by a homogeneous, amorphous material which stained pink with hematoxylin and eosin. This amyloid was deposited in the media of the vessels and resulted in marked decrease in the caliber of the lumina of many of the affected vessels. The small vessels of the heart and lungs were most severely

* These data are based on a study of material in the Section on Pathologic Anatomy of the Mayo Clinic. The study was made under the guidance of Dr. J. W. Kernohan.

involved, but the vascular amyloid was prominent in all tissues examined. In the myocardium there were areas where a moderate amount of the substance had been deposited on the reticulum between muscle bundles and nutrient capillaries. Variable degrees of myocardial atrophy had occurred in these areas. Parenchymal involvement was prominent in the liver; the hepatic cords were markedly replaced by amyloid which had been laid down on the reticulum between the sinusoids and the cords of hepatic cells. The spleen was involved diffusely and severely. The lymph nodes were affected to such a degree that the normal architecture was barely recognizable. Lipoid tissue in association with lymph nodes and elsewhere showed a thin deposit of amyloid on the surface of individual fat cells. The gallbladder was moderately thickened by masses of the material in the subepithelial tissue. The gastro-intestinal tract, genito-urinary tract, and pancreas showed involvement of only the small blood vessels. There was a small amount of amyloid between the thyroid acini. Mild involvement of the vessels in the striated muscle was noted. In some areas amyloid was surrounded by giant cells of foreign body type and by lymphocytes. Sections of marrow from the vertebrae and ribs showed no abnormality.

Case 2

A white man, 73 years of age, registered at the Clinic on January 19, 1940, with a 6 months' history of dyspnea on exertion, edema of the ankles, palpitation, and anorexia. Two months previously, when he had been hospitalized for congestive cardiac failure, an electrocardiogram showed a rate of 81 per minute, incomplete left bundle-branch block (QRS, 0.12 second), inverted T_1, delayed atrioventricular conduction (P-R, 0.24 second) and diphasic P_3. A small, high-grade epithelioma of the base of the tongue had been successfully treated with radium in 1925. In May, 1939, his blood pressure had been 140/66 mm. of Hg.

When admitted to the Clinic on January 19, 1940, the blood pressure was 109/65 mm. of Hg. Râles were heard over the bases of the lungs and the liver was palpated 4 cm. below the right costal margin. A flocculation test for syphilis gave negative results. Albuminuria was graded 2, and the blood urea level was 52 mg. per 100 cc. After rest in bed and the administration of diuretics, a transurethral prostatic resection for urinary obstruction was done on January 22. After prostatic resection, the systolic blood pressure remained under 100 except on one occasion when it rose to 110. There was continuous oliguria and the value for blood urea rose to 272 mg. per 100 cc. on February 16. On February 9, the day after the patient was given a blood transfusion of 250 cc., he became icteric. The level of direct serum bilirubin rose to 10 mg. per 100 cc. Marked dependent edema developed and the patient died on February 17, 1940.

At necropsy, dependent edema and icterus were noted. The peritoneal cavity contained about 1500 cc. of clear, yellow fluid. The visceral and parietal pericardial surfaces were lined by yellow, fibrinous material. The heart weighed 590 gm., and all of its chambers were

moderately dilated. There was moderate coronary atherosclerosis. The spleen weighed 485 gm.; the liver, 2300 gm. They were firmer than is normal. The kidneys weighed 342 gm.

All of the organs examined, except the bone marrow and spinal cord, showed vascular amyloid infiltration similar to that seen in case 1. The media of the small arteries and arterioles was the site of deposition. The most significant involvement was in the myocardium where many of the small vessels were affected. There was also severe, diffuse deposition of amyloid on the reticulum of the myocardium and atrophy of associated muscle fibers. In the gastro-intestinal tract there was amyloid in the walls of the small vessels. Some of these vessels showed resultant narrowing of their lumina. The intestinal mucosa was slightly infiltrated. In the liver there was considerable involvement of small arteries, some of which showed moderate narrowing. There was atrophy of hepatic cells in the peripheral portions of the hepatic lobules with condensation of the reticulum in these areas. In this condensed reticulum there was a small amount of poorly staining amyloid. There was slight to moderate deposition of amyloid in the walls of the small vessels of the kidneys, pancreas, spleen, adrenal glands, lymph nodes, thyroid, prostate, and seminal vesicles. In the stroma of the prostate and thyroid there were moderate, diffuse amyloid deposits. There was a thin layer of the material beneath the epithelium of the tongue. Sections of the bone marrow appeared normal. It is noteworthy that tissue taken for biopsy of the original tumor of the tongue showed no amyloid. A very small area of adenocarcinomatous tissue without suppuration was noted in the microscopic sections of the posterior portion of the prostate. It is extremely unlikely that the cured carcinoma of the tongue or the early carcinoma of the prostate could be considered causes of amyloidosis, especially since distribution of the amyloid was of the primary type.

Case 3

A white man, 56 years old, registered at the Clinic on May 4, 1943, because of a loss of 50 lb. (22.7 kg.) in the preceding year, anorexia for 3 months, jaundice for 2 months, and generalized pruritis and clay-colored stools for 10 days. In February, 1943, he had undergone transurethral prostatectomy. For 18 months he had noted dyspnea on exertion. He was moderately icteric and had "spider" angiomas on the head and neck. Axillary, cervical, and inguinal nodes were enlarged. The liver and spleen were enlarged. There was dependent edema. Râles were heard over the bases of the lungs. The blood pressure was 130/70 mm. of Hg. A flocculation test for syphilis gave negative results. The specific gravity of the urine was 1.010. Albuminuria was graded 4. The value for blood urea was 76 mg. per 100 cc.; for creatinine, 3.6 mg.; for direct bilirubin, 5.4 mg.; for indirect bilirubin, 3.0 mg.; for plasma proteins, 7.8 gm.; the albumin-globulin ratio was 1:1.47, and the prothrombin time (Quick) was 25 seconds (normal, 18 seconds). An electrocardiogram showed

a rate of 82, sinus rhythm, notched QRS_3, left axis deviation, notched P_3, inverted T_1, and diphasic T_2. Scattered cutaneous ecchymoses, shifting dullness in the abdomen, and stupor developed. The blood urea rose to 92 mg.. Death occurred on May 10, 1943.

At necropsy, moderate jaundice and edema were found. The peritoneal cavity contained about 1 l. of serosanguineous fluid. The right pleural cavity contained about 1 l., and the left about 500 cc., of clear, yellow fluid. The heart weighed 560 gm. The spleen weighed 975 gm. and was soft. The liver weighed 4000 gm., and its consistency was normal. The kidneys weighed 538 gm. There was slight enlargement of the lymph nodes throughout the body.

Microscopic examination revealed diffuse, severe infiltration of the spleen with amyloid. The hepatic parenchyma was diffusely infiltrated, and the adrenal cortex and renal glomeruli were severely involved. There was moderate infiltration around the renal tubules. Throughout the ventricles and atria there was slight to moderate infiltration with amyloid which was deposited on the reticulum surrounding the muscle fibers. In some areas there was mild atrophy of associated myocardial fibers. A small amount of the material was present beneath the endocardium of the auricles and in the tricuspid valve. With the exception of the skin and aorta, there was mild to moderate involvement of the small vessels of all tissues examined. A slight, diffuse deposition of amyloid was found in the smooth muscle of the gastro-intestinal tract. The lymph nodes were moderately and diffusely infiltrated. There was a small amount of amyloid in the alveolar walls of the lung and on the reticulum of striated muscle. The interstitial tissue of the thyroid, prostate, and testes contained considerable amyloid. There was slight thickening of the walls of a few capillaries in the bone marrow due to the deposition of amyloid. In several locations the amyloid appeared in adipose connective tissue where it was on the surface of individual fat cells. The bone marrow showed no evidence of myeloma.

Case 4

A white man, 67 years of age, registered at the Clinic on December 1, 1943, with the complaint of pain in the left side of the chest on exertion during the previous 4 years. For 9 months he had had severe dyspnea, dependent edema, and weakness which confined him to bed most of the time. Three weeks before admission, abdominal swelling, nausea and vomiting had developed. When examined he was dyspneic. Râles were heard over the bases of the lungs. Heart tones were distant. The blood pressure was 102/72 mm. of Hg. There was questionable ascites, and moderate pitting edema of the feet, ankles, and abdomen. Albuminuria was graded 2. The hemoglobin was 15.8 gm.; leukocytes, 7,600. The value for blood urea was 76 mg. and the creatinine, 2.7 mg. A flocculation test for syphilis gave negative results. A roentgenogram of the chest showed fluid in the left base. An electrocardio-

gram disclosed an auricular rate of 136 and a ventricular rate of 80, slurred QRS_1 and QRS_2, notched QRS_3, low amplitude QRS in leads I, II, and III, right axis deviation and indefinite T waves. The sedimentation rate was 2 mm. in 1 hour. The value for blood urea rose to 122 mg. and for creatinine to 3.9 mg. Anasarca became severe, and death occurred on December 15, 1943.

At necropsy about 2500 cc. of cloudy, yellow fluid were found in the peritoneal cavity. The right pleural cavity contained about 1000 cc., and the left pleural cavity about 1500 cc., of similar fluid. There were about 300 cc. of clear, yellow fluid in the pericardial sac. The heart weighed 662 gm. The spleen weighed 667 gm. and was firmer than is normal. The liver weighed 1865 gm. and showed chronic passive congestion. The kidneys weighed 304 gm.

Microscopic examination revealed infiltration of most of the organs with amyloid. The heart showed moderate to severe involvement of the ventricular myocardium and slight involvement of the auricles. The walls of the small vessels of the heart and the subendocardial tissue of the atria contained small amounts of the substance. The small vessels of the lungs contained moderate, and the alveolar walls slight, amounts of amyloid. The adrenals and spleen showed marked infiltration and the renal glomeruli moderate infiltration. The walls of the small blood vessels of every organ of the neck, chest, and abdomen showed slight to moderate amyloid infiltration. The hepatic parenchyma was uninvolved. Fat cells in several locations were surrounded by "amyloid rings." A thin collar of amyloid surrounded the acini of Brunner's glands. The thyroid acini were separated by moderate amounts of the substance. Vessel walls in the testes contained amyloid. The diaphragm showed involvement similar to that of the heart. In several sites the amyloid was surrounded by a foreign body reaction consisting of giant cells and a few lymphocytes. A section of vertebral marrow appeared normal.

Case 5

A white woman, 44 years old, who registered at the Clinic on October 9, 1944, had had edema of the lower extremities and abdomen for 7 months. In the past 3 months she had noted abdominal swelling and had had one paracentesis abdominis. Recently, marked weakness and slight dyspnea had developed. The blood pressure was 110/80 mm. of Hg. Breath sounds were diminished over the lower lung fields. The abdomen was markedly distended and the lower parts of the trunk and legs were edematous. Albuminuria was graded 2 to 4. The hemoglobin was 12.1 gm.; erythrocytes, 4,130,000; and leukocytes, 9,400. The electrocardiogram showed a rate of 72 per minute, low amplitude QRS in leads I, II, and III, low amplitude T_2 and inverted T_3. The blood urea level was 24 mg. The serum protein level was 4.2 gm., with an albumin-globulin ratio of 1.0:1.4. One hour after injection of 5 mg. of bromsulfalein per kg. of body weight, more than 40 per cent of the substance remained in the blood stream. On October 12, 5000 cc. of cloudy peritoneal fluid were removed. Persistent oliguria began and did not respond

to saline and mercurial diuretics. On October 23, the value for blood urea had risen to 192 mg. and for creatinine, to 9.3 mg. She had extreme drowsiness beginning on October 19, and on this date the blood sugar level was 35 mg. per 100 cc. Only slight improvement followed intravenous administration of glucose. Coma supervened, and death occurred on October 24, 1944.

At necropsy there was brownish yellow discoloration of the skin. Evidence of residual ascites was present. The heart weighed 220 gm. The spleen weighed 480 gm. and was firm. The liver weighed 2800 gm. and was firmer than is normal. There were scattered submucosal petechiae throughout the gastro-intestinal tract. The kidneys weighed 427 gm.

Microscopic examination revealed marked amyloid infiltration of the liver, spleen, kidneys, and adrenal glands. The hepatic parenchyma was almost completely replaced with amyloid. Renal glomerular infiltration left little space for blood flow through the capillaries. The splenic involvement was diffuse or "bacony" in type. Throughout the myocardium there was moderate, diffuse amyloid infiltration, with deposition on the reticulum. There was patchy atrophy of associated muscle fibers. A thin layer of amyloid lay beneath the endocardium of the auricles. The small vessels of the heart, as well as of every organ of the chest and abdomen, showed slight to moderate thickening of the media as a result of the presence of amyloid. There was slight infiltration of the pulmonary alveolar walls. Several lymph nodes and the gastric mucosa showed slight diffuse involvement. The fat in the region of the pancreas and in the epicardium showed a thin layer of amyloid surrounding individual cells. The bone marrow was normal grossly.

Case 6

A white man, 40 years of age, registered at the Clinic on November 9, 1947, because of edema of the ankles and ascites of 6 weeks' duration. In July, 1947, he had had paresthesia with slight swelling of the hands and forearms. Two months later he began having dyspnea on exertion and noted pain in the back. On examination, the blood pressure was 102/80 mm. of Hg.; pulse, 120. The tongue was red and the submaxillary glands were large. Moist râles were heard over the bases of the lungs. The liver was enlarged and the abdomen distended. There was marked edema of the lower extremities. The patient died suddenly before laboratory study was made.

At necropsy there was marked edema. The peritoneal cavity contained about 4000 cc. of clear, amber fluid. The right pleural cavity contained about 300 cc., and the left about 200 cc., of similar fluid. The heart weighed 525 gm. and was very firm. Two small mural thrombi were found in the right auricular appendage. The spleen weighed 535 gm. and was very firm. Its cut surface was homogeneous, grayish purple, and showed no markings. The liver weighed 2485 gm., and the

centers of the lobules were dark. The wall of the stomach was somewhat thicker than is normal. The tongue was moderately enlarged as were the submaxillary and sublingual salivary glands. The right kidney weighed 165 gm. and its pelvis contained a small amount of inspissated, sandy material. The left kidney weighed 120 gm. Its parenchyma was thin over dilated calyces which, along with the renal pelvis and the dilated left ureter, contained inspissated, sandy material. The heart and spleen showed the characteristic mahogany brown color on application of iodine.

Microscopic study of the heart revealed marked infiltration of the myocardium of all four chambers by a homogeneous material which stained pink with eosin. There was marked associated atrophy of muscle fibers (Fig. 1). The spleen was diffusely infiltrated with amyloid to the extent that normal architecture was barely recognizable. The tongue showed infiltration between its mucous glands and muscle fibers (Fig. 2). The connective tissue between the lobules of the submaxillary and sublingual glands was markedly thickened as a result of amyloid deposits, and smaller amounts of interacinar amyloid were seen. The pancreas presented a similar picture. In the esophagus and stomach there was mild infiltration of the muscularis. The gastric mucosa contained a small amount of the substance. There was slight patchy infiltration in the lungs, chiefly in the vicinity of the bronchi. The thyroid was slightly involved. The wall of the urinary bladder and the prostate contained small amounts of amyloid between the muscle bundles. Lymph nodes from several sites showed slight amyloid infiltration and several lymph nodes showed none. Adipose connective tissue from several locations contained amyloid which surrounded individual fat cells. In the left kidney there was evidence of hydronephrotic atrophy and healed pyelonephritis. There was no current, active, inflammatory process in the kidneys that could be considered as the cause of amyloidosis. In addition, the amyloid distribution was not of the secondary type. The amyloid in this case showed only very slight metachromasia with the methyl violet stain but stained well with Congo red in some locations. No evidence of myeloma was found in sections of the vertebral marrow, but one section contained a lymph follicle which was slightly infiltrated with amyloid.

Comment

In these 6 examples of systemic amyloidosis there were no demonstrable preceding or etiologic diseases. The staining reactions of the material were similar in all instances, although they were much more

variable than in secondary amyloidosis. The amyloid was homogeneous and amorphous and stained pink with hematoxylin and eosin. In all instances there was definite metachromasia in at least some of the sections stained with methyl violet, although the intensity of the reddish violet color varied markedly in different cases. With the Congo red stain the amyloid assumed a red color, but this stain was not as satisfactory as methyl violet in differentiating amyloid from dense connective tissue. For this differentiation the van Gieson stain was extremely valuable. With it the amyloid stained yellow or very faintly pink, whereas hyaline or collagenous connective tissue of similar density stained brilliant red.

It is apparent that the distribution of amyloid in these cases differs from that usually described for secondary amyloidosis, especially in the constancy of involvement of the heart and the frequency of involvement of striated muscle, lungs, and small blood vessels throughout the body. The cases in which involvement of the spleen, adrenals, liver, and kidneys occurred demonstrate, however, that amyloid distribution of the so-called secondary type is overlapped in primary systemic amyloidosis.

The heart contained significant amounts of the material in all 6 examples of primary systemic amyloidosis. Grossly, the nature of the disease was not always evident. In 2 cases, small, translucent, subendocardial masses of amyloid were seen. The infiltration in the hearts was diffuse, involving the myocardium of the auricles and ventricles. It consisted chiefly of two main types. Patchy or diffuse deposition of amyloid on the reticulum surrounding the muscle bundles was associated with partial atrophy of the encased muscle fibers (Fig. 1). Amyloid was deposited also in the walls of the small arteries, arterioles, and veins of the myocardium, sometimes markedly decreasing their lumina. A small amount of amyloid was found beneath the atrial endocardium in all 6 hearts. Small masses of amyloid were found within the tricuspid valve in case 3. In only 2 cases was there any significant coronary atherosclerosis and this was moderate. The vessels and fat of the epicardium contained variable but small amounts of amyloid.

The hearts were larger than is normal except in case 5. The average cardiac weight was 505 gm. Grossly, they showed concentric hypertrophy. This hypertrophy occurred in patients whose blood pressure was normal or subnormal. The highest pressure (140/80 mm. of Hg.) was observed in case 1. It appears that amyloidosis produces large hearts in the absence of valvular defects or hypertension. There were low amplitude QRS complexes in the electrocardiograms of 3 cases.

Cardiac decompensation supplied the main clinical signs and symptoms as well as the cause of death in 4 patients.

The involvement of small arteries, arterioles, and small veins of many organs was a prominent feature in several of these cases. The material was deposited in the media. In mild degrees of the process it could be seen as a thin layer surrounding the smooth muscle cells. As the disease progressed, the muscle cells became separated by the material and some decrease in size of the vascular lumen resulted (Fig. 3). With severe involvement of the vessel, the lumen was markedly narrowed by the thickened media and amyloid often extended into the adventitia.

The liver showed marked parenchymal amyloid infiltration in 2 cases. This resulted in severe atrophy of the hepatic cords (Fig. 4). The material was deposited on both sides of the reticulum fibers lying between the sinusoids and the cords of liver cells. The exact site of deposition in the liver and in the heart was demonstrated by staining sections for reticulum and then with methyl violet. In one case only slight, and in another moderate, amounts of amyloid were present in the hepatic parenchyma. In only 2 cases was the hepatic parenchyma free of amyloid. The small vessels, especially the arteries, of the portal triads showed slight to severe infiltration of their walls in all cases. The livers in the cases of moderate or severe amyloidosis were firm and rubbery and had an average weight of 2880 gm.

In case 5, with severe hepatic amyloidosis, a clinical diagnosis of cirrhosis of the liver was entertained. A bromsulfalein test of liver function revealed retention of more than 40 per cent of the dye at the end of 1 hour. In case 3, in which there was moderate involvement of the liver, obstructive jaundice was the clinical diagnosis and the direct serum bilirubin rose to 5.4 mg. and the indirect to 3.0 mg. These two patients had jaundice, ascites, and edema of the legs. In case 3, the firm, enlarged liver was noted clinically. The clinical picture was complicated by severe renal amyloidosis with marked proteinuria in these 2 cases. In both there was a reversed albumin-globulin ratio with blood protein levels of 7.8 gm. and 4.24 gm., respectively, and there was considerable terminal azotemia.

Involvement of the small blood vessels of the kidneys was noted in 5 cases. In 2 patients whose signs and symptoms suggested primary hepatic disease, the glomerular amyloidosis was severe, that is, the diameter of the glomerular capillary lumina was greatly decreased. These patients had marked albuminuria and the changes in blood protein mentioned above. In these 2 patients the terminal levels of blood urea were 92 and 192 mg., respectively. The coexisting hepatic and

cardiac disease made it difficult to evaluate the clinical significance of the renal lesion. In the remaining 4 patients, deposit of glomerular amyloid was moderate in one, mild in one, and absent in 2.

The spleen was severely infiltrated with amyloid in 5 instances. Diffuse "bacony" involvement of the splenic cords with loss of splenic corpuscles had occurred (Fig. 5). These spleens were firm and their average weight was 560 gm. Again, portal obstruction and cardiac insufficiency may have affected their size. Slight splenic amyloid infiltration was noted in the remaining case. Rather slight involvement of the walls of the small blood vessels was seen in all of the spleens.

The gastro-intestinal tract was slightly to moderately involved in all 6 cases. The small blood vessels were constantly affected. In all cases, amyloid was deposited diffusely, in slight to moderate degree, on the smooth muscle cells of the muscularis mucosae and muscularis propria. Mild involvement of the mucous membrane was seen in only 3 cases. No gross alterations of the gastro-intestinal tract due to amyloid were observed. Gastro-intestinal symptoms, such as constipation, diarrhea, anorexia, and vomiting, occurred in 4 cases, but, again, the problem of evaluation in the face of coexisting cardiac, hepatic, and renal disease is obvious.

Amyloid deposition of moderate to severe degree was observed in or around the small vessels of the lungs in 5 cases (Fig. 6). Slight patchy deposits around the capillaries of the alveolar walls were observed in 4 cases. In one case no amyloid was found in the lungs. In none was the pulmonary amyloidosis of apparent clinical importance.

The small vessels around and in the lymph nodes were slightly to severely affected in all but one case (Fig. 7). Diffuse infiltration of the nodes similar to that seen in the "bacony" spleens occurred in 5 cases and, in 2 of these, it was responsible for clinically evident enlargement of the cervical nodes. The diagnostic value of biopsy of lymph nodes should be borne in mind.

There was slight to moderate infiltration of the walls of the small blood vessels in striated muscle in the 5 cases in which samples were available for study. In 3 of these there was a small amount of amyloid laid down on the reticulum surrounding the muscle fibers. No definite symptoms can be ascribed to the infiltration in the muscle, but it does serve to emphasize the diffuse deposition in primary systemic amyloidosis.

The adrenal cortex was largely replaced with amyloid in 3 cases. The deposition was similar to that seen in secondary amyloidosis. The periadrenal and medullary small vessels in 5 cases were affected. Peri-

adrenal fat was infiltrated in the sixth case. Signs pointing to adrenal insufficiency were not observed.

The blood vessels of the thyroid were involved in 4 of the 5 cases in which the gland was studied. Diffuse infiltration between the acini occurred in all 5 (Fig. 8). In one of these the acini were widely separated, and in the others moderately separated, by the substance.

In none of these 6 cases was macroglossia noted clinically, although the tongue was found to be enlarged at necropsy in one. In only 2 instances was the tongue examined at necropsy. These showed microscopic evidence of moderate amounts of amyloid in the small vessels, around some of the mucous glands, and on the reticulum surrounding some of the muscle fibers.

The walls of the blood vessels in the pancreas were involved in all but one instance. In 4 of the 6 cases, amyloid was found in the pancreatic interstitial tissue. In the 4 men the small prostatic vessels contained amyloid in their walls. In 3 instances, moderate diffuse infiltration in the smooth muscle of the gland was seen. In all 6 cases, amyloid was seen in the adipose tissue of such sites as the epicardium, pancreas, and periadrenal regions. The amyloid appeared to be laid down upon the surface of the fat cells, giving their walls a thickened appearance. These rings of amyloid on fat cells have been considered characteristic of primary amyloidosis.

No skin lesions were observed clinically except purpura, which occurred in one patient. Sections of skin from the trunk were studied in 3 cases and no abnormality was noted. The cause of the purpura was not determined. Amyloid infiltration of small blood vessels of the skin has been held responsible for the purpura by some observers.[2]

Summary and Conclusions

The 6 cases of primary systemic amyloidosis reported in this paper demonstrate the remarkably diffuse involvement of mesodermal structures which occurs in this disease. The organs most severely affected in secondary amyloidosis are, however, not immune to infiltration in the primary type.

The clinically important lesions were cardiac in 3 cases, which is in conformity with the findings in the majority of reported cases of primary systemic amyloidosis.

An unusual syndrome was presented in the 2 cases in which primary hepatic disease was simulated. The association of signs of hepatic disease and of renal disease in these is noteworthy.

That macroglossia as a diagnostic mainstay in primary systemic

amyloidosis has been overrated is evidenced by its absence in 5 of these cases. It was observed in less than one-third of the cases reported in the literature.

The identity of the homogeneous, amorphous, pink-staining material seen in sections stained with hematoxylin and eosin should be confirmed by the use of special stains. In these 6 cases the amyloid showed definite metachromasia with methyl violet. The affinity of amyloid for Congo red in primary amyloidosis was not as great as that observed in secondary amyloidosis. The van Gieson connective tissue stain is valuable in differentiating amyloid from hyaline or collagenous connective tissue of similar density.

Biopsy of affected organs is the only certain diagnostic procedure in the cases in which the amyloid lacks affinity for intravenously injected Congo red.

No common denominator in these cases furnished any clue as to the pathogenesis of primary systemic amyloidosis.

REFERENCES

1. Wild, C. Beitrag zur Kenntnis der amyloiden und der hyalinen Degeneration des Bindegewebes. *Beitr. z. path. Anat. u. z. allg. Path.*, 1886, **1**, 177–199.
2. Lubarsch, O. Zur Kenntnis ungewöhnlicher Amyloidablagerungen. *Virchows Arch. f. path. Anat.*, 1929, **271**, 867–889.
3. Koletsky, S., and Stecher, R. M. Primary systemic amyloidosis. Involvement of cardiac valves, joints and bones, with pathologic fracture of the femur. *Arch. Path.*, 1939, **27**, 267–288.
4. Lindsay, S., and Knorp, W. F. Primary systemic amyloidosis. *Arch. Path.*, 1945, **39**, 315–322.
5. Lindsay, S. The heart in primary systemic amyloidosis. *Am. Heart J.*, 1946, **32**, 419–437.
6. Brown, H. A., and Selzer, G. A case of primary amyloidosis. *Clin. Proc.*, 1944, **3**, 227–238.
7. Golden, A. Primary systemic amyloidosis of the alimentary tract. *Arch. Int. Med.*, 1945, **75**, 413–416.
8. Bannick, E. G., Berkman, J. M., and Beaver, D. C. Diffuse amyloidosis. Three unusual cases: a clinical and pathologic study. *Arch. Int. Med.*, 1933, **51**, 978–990.
9. Pick, L. Über atypische Amyloidablagerung. *Klin. Wchnschr.*, 1931, **10**, 1515.
10. Barnard, W. G., Smith, F. B., and Woodhouse, J. L. Atypical amyloidosis with macroglossia. *J. Path. & Bact.*, 1938, **47**, 311–314.
11. Kernohan, J. W., and Woltman, H. W. Amyloid neuritis. *Arch. Neurol. & Psychiat.*, 1942, **47**, 132–140.
12. Bürümcekci, K. Mitteilung eines Falles von atypischer Amyloidose (Paramyloidose). *Virchows Arch. f. path. Anat.*, 1938, **302**, 607–617.
13. Fowler, W. M. Idiopathic amyloidosis. *J. Iowa M. Soc.*, 1936, **26**, 98–100.

14. Soisalo, P., and Ritama, V. Zur atypischen Amyloidose mit besonderer Berücksichtigung des Herzens. *Acta med. Scandinav.*, 1943–44, **116**, 260–272.

15. Ferris, H. W. Amyloidosis of lungs and heart. *Am. J. Path.*, 1936, **12**, 701–718.

16. Bell, A. W. Amyloid infiltration: report of a case. *Journal-Lancet*, 1922, **42**, 306–308.

17. Eisen, H. N. Primary systemic amyloidosis. *Am. J. Med.*, 1946, **1**, 144–160.

18. Ranström, S. Amyloidosis myocardii. *Acta med. Scandinav.*, 1945–46, **123**, 111–125.

19. Steinhaus, F. Ueber eine seltene Form von Amyloid- und Hyalin- Infiltration am Circulations- und Digestionsapparat. *Ztschr. f. klin. Med.*, 1902, **45**, 375–384. (Cited by Koletsky and Stecher.[8])

20. Gerstel, G. Über atypische Lokalisation des Amyloids, insbesondere über die Makroglossia amyloides diffusa. *Virchows Arch. f. path. Anat.*, 1932, **283**, 466–488. (Cited by Koletsky and Stecher.[8])

21. Pearson, B., Rice, M. M., and Dickens, K. L. Primary systemic amyloidosis. Report of two cases in Negroes, with special reference to certain histologic criteria for diagnosis. *Arch. Path.*, 1941, **32**, 1–10.

22. Gerber, I. E. Amyloidosis of the bone marrow. *Arch. Path.*, 1934, **17**, 620–630.

23. Götze, W., and Krücke, W. Über Paramyloidose mit besonderer Beteiligung der peripheren Nerven und granulärer Atrophie des Gehirns, und über ihre Beziehungen zu den intracerebralen Gefässverkalkungen. *Arch. f. Psychiat.*, 1941–42, **114**, 183–213.

24. De Navasquez, S., and Treble, H. A. A case of primary generalized amyloid disease with involvement of the nerves. *Brain*, 1938, **61**, 116–128. (Cited by Kernohan and Woltman.[11])

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 15

FIG. 1. Case 6. Heart muscle, showing diffuse infiltration with amyloid and resultant atrophy of muscle fibers. Hematoxylin and eosin stain. × 55.

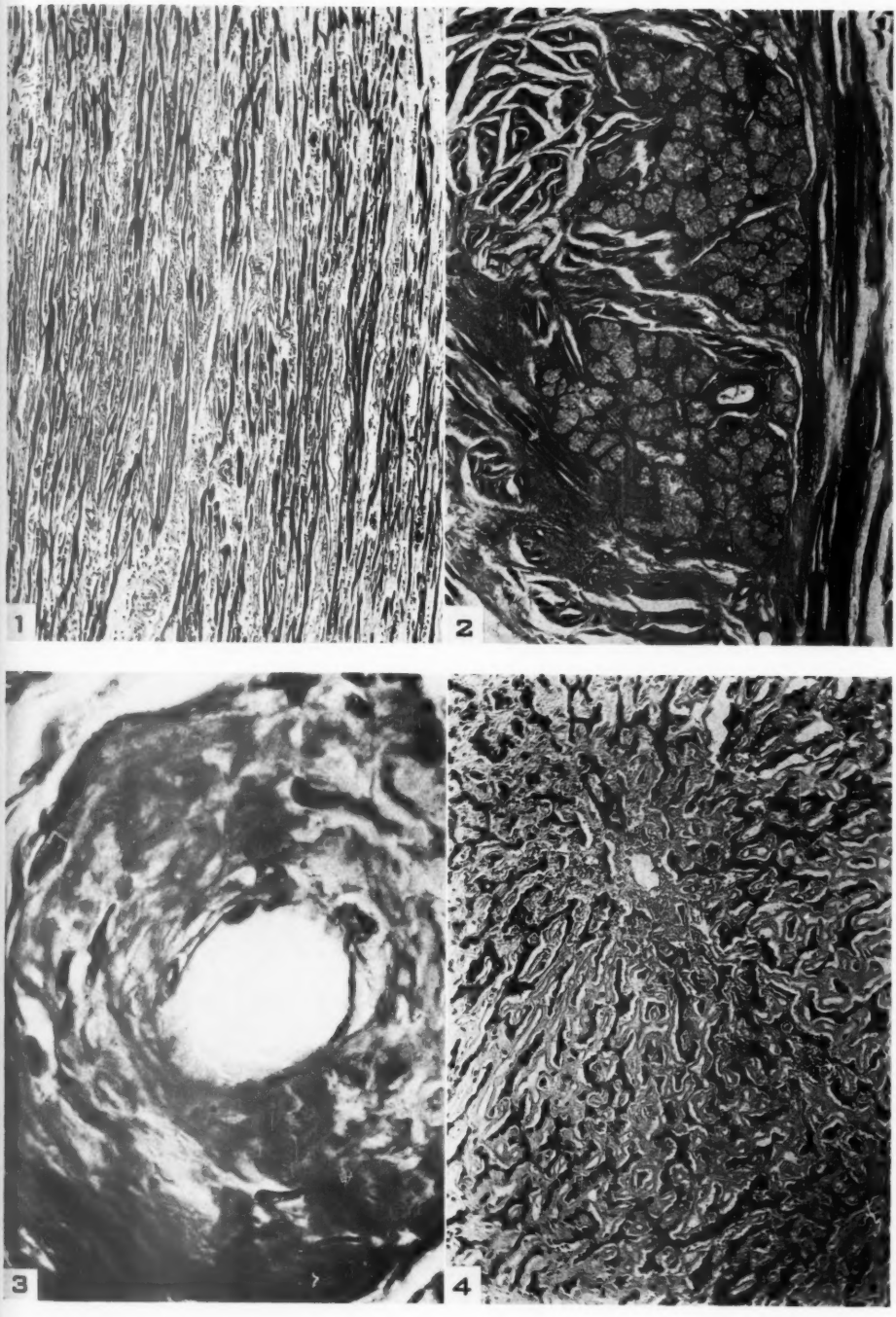
FIG. 2. Case 6. Tongue, showing the paler staining amyloid between the mucous glands and the muscle bundles. Hematoxylin and eosin stain. × 55.

FIG. 3. Arteriole, showing amyloid infiltration of the media with separation of the smooth muscle cells. Hematoxylin and eosin stain. × 675.

FIG. 4. Hepatic lobule, showing marked atrophy due to amyloid infiltration. Hematoxylin and eosin stain. × 85.

1

3



Dahlin Primary Amyloidosis

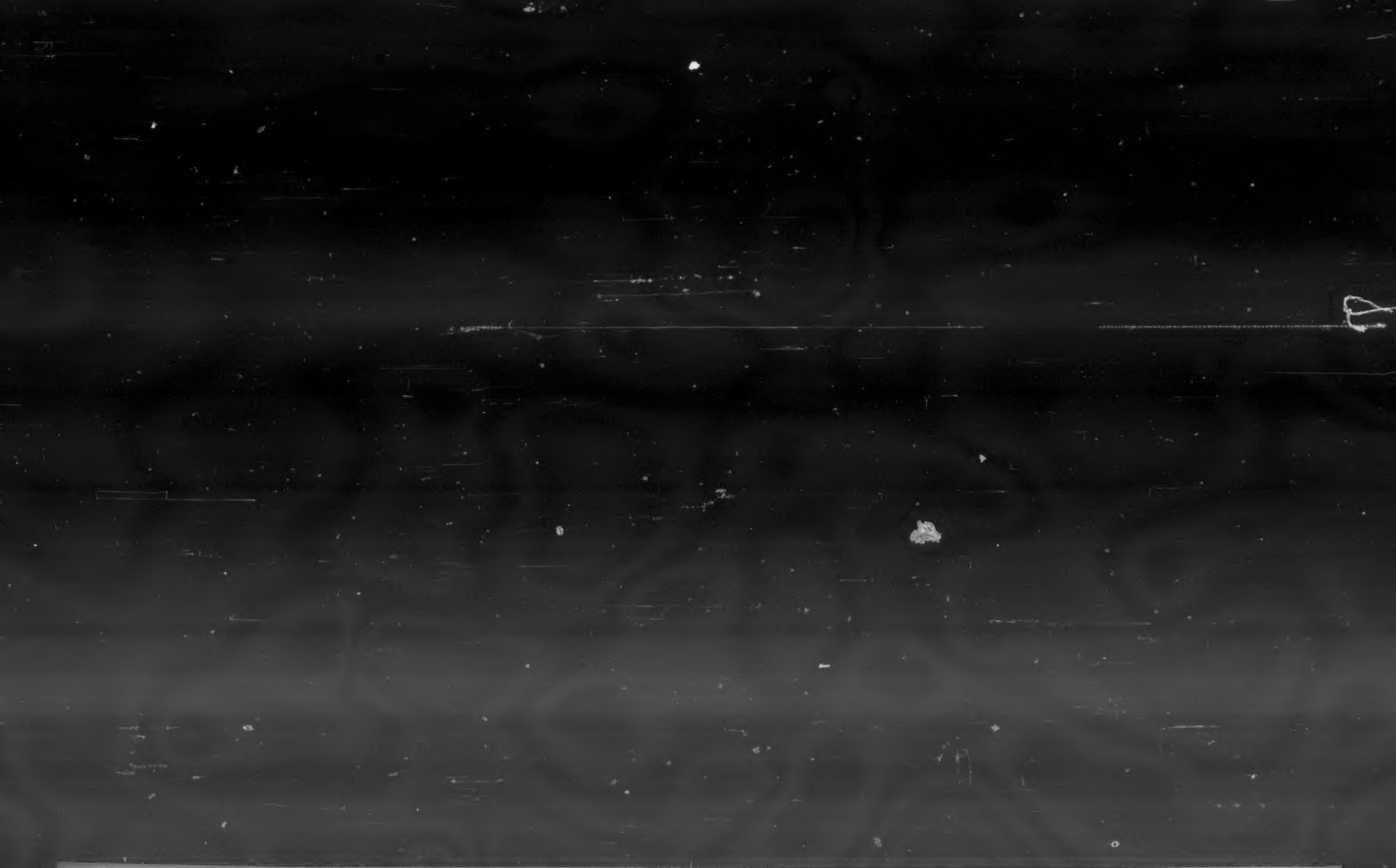
Plate 16

Fig. 5. Spleen, showing diffuse infiltration with amyloid. Hematoxylin and eosin stain. × 55.

Fig. 6. Lung. Dark areas represent amyloid. Photographed using B and H Wratten filter. Methyl violet stain. × 60.

Fig. 7. Lymph node markedly infiltrated. Amyloid surrounds the perinodal fat cells. Hematoxylin and eosin stain. × 110.

Fig. 8. Thyroid with moderate amyloid infiltration between acini. Hematoxylin and eosin stain. × 130.



Dahlin Primary Amyloidosis

THE TOPOGRAPHY OF CHRONIC GASTRITIS IN OTHERWISE NORMAL STOMACHS *

ROBERT HEBBEL, M.D.

(From the Department of Pathology, University of Minnesota, Minneapolis, Minn.)

Most observers are agreed that the changes in the gastric mucosa which, individually and together, constitute so-called chronic gastritis are frequently encountered in adults (Hillenbrand,[1] Faber,[2] Guiss and Stewart,[3] Hebbel [4]). While it is recognized that such deviations from the normal may be from focal to diffuse in distribution, determinations of the incidence of gastritis in otherwise normal stomachs have referred largely to the qualitative features of the process and there is uncertainty as to the frequency with which varying distributions of the changes may be found. That varying distributions do exist was emphasized by Hillenbrand in a detailed study of 21 stomachs from patients over 35 years of age. As part of a study of the relationship between chronic gastritis and gastric cancer it seemed desirable to have more information concerning the topography of gastritis in stomachs otherwise without disease. Gastritis, commonly found with cancer of the stomach, may vary in its extent (Hebbel) and the significance of that variation can be interpreted only in relation to similar processes found in stomachs without cancer. This paper presents the results of a survey of a group of stomachs with reference to the distribution of the gastritic changes encountered.

MATERIALS AND METHODS

The material consisted of stomachs obtained at autopsy from persons of both sexes and all ages in whom death resulted from a wide variety of causes and whose past histories recorded no gastro-intestinal complaints. Cases of pernicious anemia were excluded. The material was otherwise selected only by the exclusion of specimens with ulcer, scar, or tumor in either the stomach or duodenum and by further exclusion, after microscopic examination, of those specimens with obscuring postmortem changes.

Strips of mucosa were removed from the entire lesser and greater curvatures of each specimen and made into rolls of convenient size from which blocks were cut and embedded in paraffin. Sections were prepared in the usual manner. By this means the entire length of the

* This study was made possible by a grant-in-aid from the National Advisory Cancer Council, United States Public Health Service.

Received for publication, February 26, 1948.

Read by title at the Forty-Fifth Annual Meeting of The American Association of Pathologists and Bacteriologists, Philadelphia, March 12 and 13, 1948.

stomach on each curvature was examined. From about half of the specimens similar preparations were made from mid-anterior and mid-posterior walls. It had been anticipated that differences might exist, but the findings were so constantly similar to those of the curvatures that preparation of these additional sections was discontinued.

The sections were searched for deviation from the usually accepted normal structure. The several features included in the picture of chronic gastritis are reasonably well established and have been dealt with in many reports (Konjetzny,[5] Hillenbrand,[1] Faber,[2] Magnus,[6] Guiss and Stewart,[3] and many others). Of these, only the following have been included in the accompanying tables (I to VII): Cellular infiltration, lymph follicles, atrophy, intestinal metaplasia, pseudopyloric glands, cystic glands, and erosions. Other accompanying features such as fibrosis, thickening of the muscularis mucosae, Russell's corpuscles, and heterotopic glands have been omitted. To indicate roughly the relative severity or extent, each of the changes, when present, was graded from 1 to 3.

Infiltration refers to the free cells, chiefly lymphocytes, in the stroma. A normal number is not established but there is general agreement (Hillenbrand,[1] Konjetzny,[5] Kalima[7]) that the diagnosis of chronic gastritis should seldom rest on cellular infiltration alone. In this material, infiltration was graded as follows: If free cells were scant and inconspicuous, they were considered to be absent. Grade 1 infiltration indicates a mild degree which was arbitrarily considered within normal limits. Grade 2 indicates moderate, and grade 3 severe, infiltration. There are no sharp divisions between these categories. Few would question that severe infiltration is abnormal and few would insist that a mild degree of infiltration is significant, but there may be difference of opinion as to the proper designation of intermediate degrees. For the purposes of this paper, infiltration in excess of grade 1 has been considered abnormal. In the main, excess infiltration was found in conjunction with other changes and was, with few exceptions, uniform throughout the segment considered. In a few specimens, excess infiltration alone was present and these were kept in a separate category. That such specimens deviate from the ideal normal is certain, but that this probably reversible change is as significant as, or of necessity related to, parenchymal alterations may be questioned.

Lymphoid aggregates, with or without reaction centers, were graded 1 to 3 in the following manner: Grade 1 indicates 1 to 5 nodules, grade 2 indicates 6 to 12 nodules, and grade 3 indicates more than 12 nodules

for the segment of mucosa concerned. No attempt has been made to make antrum and body more comparable in this respect by correcting for the greater length of the strips of body mucosa examined. A normal number has not been established. Konjetzny [5] considered lymphoid follicles to be rare under normal conditions, while Hillenbrand [1] frequently found them in the absence of other changes. In this material, no specimen has been considered abnormal on the basis of the number of lymph nodules alone. Few were encountered in the absence of other changes.

Atrophy, referring to the loss of normal glandular structure, with or without actual thinning of the mucosa, was graded as follows on the basis of distribution and not of severity in any area: Grade 1 refers to one or two foci of microscopic size. Grade 2 refers to several or more foci or small patches. The largest isolated patches encountered rarely exceeded about 5 mm. and larger patches were associated with diffuse changes. Grade 3 refers to atrophy of greater or less degree uniformly involving a whole segment. While the severity of the process varied, it may be noted that the majority of examples of diffuse atrophy were of moderate degree.

Metaplasia refers to the presence of epithelium, similar to that of the small intestine, in crypts and glands. Grade 1 refers to one or two focal areas of microscopic size. Grade 2 refers to several such focal or slightly larger areas. Grade 3 refers to many patchy areas or uniform involvement. The more severe degrees parallel atrophy.

Cysts, referring to cystic changes in the glands, were graded as follows: Grade 1 indicates one or two cystic glands, grade 2 indicates several cystic foci, and grade 3 indicates numerous cysts.

Erosions were rare in this material and those encountered were all healed. They were graded as follows: Grade 1 indicates an isolated erosion, grade 2 indicates several, and grade 3 indicates numerous erosions.

Pseudopyloric glands were graded as follows: Grade 1 refers to one to several focal areas of microscopic size, grade 2 refers to moderate numbers, and grade 3 refers to numerous glands of this type. In the main this feature is observed in association with other changes but may exist alone.

With the exceptions noted in respect to infiltration and lymph follicles, the presence of any of the above changes placed a specimen in the abnormal group. The findings are assembled in Tables I to VII. Since there were no differences related to sex, the sex incidence of the

several changes has been omitted. With some tabulations, totals for the first and second 5 decades of life as well as for the entire series have been given to emphasize the predominance of the changes in the older age groups.

Presentation of Data

Table I shows the graded findings in each of the several categories for the lesser curvature of the antrum. Infiltration was absent in 20 and present in 77 specimens (44, grade 1; 29, grade 2; 4, grade 3). Follicles were absent in 31 specimens and were present in 66 (36, grade 1; 26, grade 2; 4, grade 3). Atrophy was absent in 61 specimens and was present in 36 (9, grade 1; 16, grade 2; 11, grade 3). Metaplasia was absent in 62 specimens and present in 35 (20, grade 1; 13, grade 2; 2, grade 3). Cysts were absent in 75 specimens and present in 22 (17, grade 1; 5, grade 2). Healed erosions, all grade 1, were present in 12 specimens.

Table II shows, in similar manner, the findings on the greater curvature of the antrum. Infiltration was absent in 21 specimens and was present in 76 (50, grade 1; 23, grade 2; 3, grade 3). Follicles were absent in 34 specimens and present in 63 (45, grade 1; 17, grade 2; 1, grade 3). Atrophy was not found in 71 specimens and was present in 26 (7, grade 1; 14, grade 2; 5, grade 3). Metaplasia was absent in 71 specimens and present in 26 (16, grade 1; 9, grade 2;, 1, grade 3). Cysts were absent in 83 specimens and present in 14 (9, grade 1; 5, grade 2). Erosions were absent in 83 specimens and present in 14 (13, grade 1; 1, grade 2).

Table III shows the graded findings for the mucosa of the lesser curvature of the body. Infiltration was absent in 29 specimens and present in 68 (37, grade 1; 26, grade 2; 5, grade 3). Follicles were not present in 33 specimens and were found in 64 (31, grade 1; 30, grade 2; 3, grade 3). Atrophy was absent in 72 specimens and present in 25 (6, grade 1; 5, grade 2; 14, grade 3). Metaplasia was absent in 79 specimens and present in 18 (7, grade 1; 11, grade 2). Pseudopyloric glands were absent in 81 specimens and present in 16 (3, grade 1; 12, grade 2; 1, grade 3). Cysts were absent in 87 specimens and present in 10 (8, grade 1; 2, grade 2). Only one specimen showed a healed erosion.

Table IV shows the findings on the greater curvature of the body. Infiltration was absent from 33 specimens and present in 64 (37, grade 1; 22, grade 2; 5, grade 3). Follicles were absent from 33 specimens and were present in 64 (32, grade 1; 29, grade 2; 3, grade 3). Atrophy was absent in 74 specimens and was present in 23 (10, grade 1; 2, grade

TABLE I

Findings, as Graded, on Lesser Curvature of Antrum

Age groups	No.	Infiltration				Follicles				Atrophy				Metaplasia				Cysts				Erosions			
		0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
1–10	6	5	1			5	1			6				6				6				6			
11–20	4	3	1			3	1			3			1	3	1			4				3	1		
21–30	11	4	4	3		6	2	3		11				10	1			10	1			9	2		
31–40	9	4	3	2		4	2	3		6	1	2		8	1			9				8	1		
41–50	14	2	8	4		3	6	5		8	3	2	1	8	4	2		9	3	2		13	1		
51–60	11		7	2	2	4	4	2	1	6	3	2		7	2	2		7	4			10	1		
61–70	21	1	9	11		4	11	3	3	11		4	6	8	6	5	2	15	5	1		19	2		
71–80	16		7	7	2	2	5	9		8	1	5	2	10	3	3		11	3	2		13	3		
81–90	4	1	3				4			1	1	1	1	1	2	1		3	1			3	1		
91–100	1		1					1		1				1				1				1			
Totals	97	20	44	29	4	31	36	26	4	61	9	16	11	62	20	13	2	75	17	5		85	12		

TABLE II

Findings, as Graded, on Greater Curvature of Antrum

Age groups	No.	Infiltration				Follicles				Atrophy				Metaplasia				Cysts				Erosions			
		0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
1–10	6	5	1			4	2			6				6				6				6			
11–20	4	3	1			3	1			3			1	3	1			4				3	1		
21–30	11	5	4	2		7	2	2		10	1			10	1			10	1			10	1		
31–40	9	5	3	1		6	3			7	1	1		8	1			9				8	1		
41–50	14	2	11	1		3	9	2		12	1	1		12	2			12	1	1		11	3		
51–60	11		8	2	1	5	3	2	1	9	1	1		9	2			7	4			10	1		
61–70	21	1	11	9		4	12	5		12	1	6	2	13	2	5	1	18	2	1		18	2	1	
71–80	16		7	7	2	2	8	6		10	1	3	2	8	5	3		14		2		14	2		
81–90	4		3	1			4			1	1	2		1	2	1		2	1	1		2	2		
91–100	1		1				1			1				1				1				1			
Totals	97	21	50	23	3	34	45	17	1	71	7	14	5	71	16	9	1	83	9	5		83	13	1	

TABLE III

Findings, as Graded, on Lesser Curvature of Body

Age groups	No.	Infiltration				Follicles				Atrophy				Metaplasia				Pseudopyloric glands				Cysts				Erosions			
		0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
1–10	6	6				6				6				6				6				6				6			
11–20	4	2	2			2	1	1		4				4				4				4				4			
21–30	11	6	3	2		7	2	2		10			1	11				10		1		11				11			
31–40	9	5	2	2		5	3	1		9				9				9				9				9			
41–50	14	4	6	4		5	4	5		12		1	1	12	1	1		12	1	1		13	1			14			
51–60	11	1	5	4	1	1	6	4		7	2	1	1	7	3	1		9		2		10	1			11			
61–70	21	2	10	7	2	2	9	7	3	11	2	3	5	14	2	5		16	2	3		17	3	1		21			
71–80	16	2	7	5	2	5	3	8		12			4	13	1	2		12		3	1	15		1		15	1		
81–90	4		2	2			2	2			2		2	2		2		2		2		2	2			4			
91–100	1	1					1			1				1				1					1			1			
Totals	97	29	37	26	5	33	31	30	3	72	6	5	14	79	7	11		81	3	12	1	87	8	2		96	1		

TABLE IV

Findings, as Graded, on Greater Curvature of Body

Age groups	No.	Infiltration				Follicles				Atrophy				Metaplasia				Pseudopyloric glands				Cysts				Erosions			
		0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
1–10	6	6				4	2			6				6				6				6				6			
11–20	4	4				3	1			4				4				4				4				4			
21–30	11	6	4	1		6	2	3		10			1	10	1			11				11				11			
31–40	9	6	2	1		5	2	2		9				9				9				8	1			9			
41–50	14	3	8	3		5	4	5		13			1	13	1			13		1		13	1			14			
51–60	11	2	5	3	1	1	5	4	1	8	2		1	9	1	1		10		1		1				11			
61–70	21	2	11	7	1	5	8	7	1	12	5	1	3	16	2	3		17	1	3		17	3	1		20	1		
71–80	16	4	5	5	2	4	6	5	1	11	1	1	3	13	1	2		12	1	2	1	15		1		15	1		
81–90	4		1	2	1		1	3			2		2	2		1	1	2		2		2	2			4			
91–100	1		1				1			1				1				1					1			1			
Totals	97	33	37	22	5	33	32	29	3	74	10	2	11	83	6	7	1	85	2	9	1	87	8	2		95	2		

2; 11, grade 3). Metaplasia was found in 14 specimens (6, grade 1; 7, grade 2; 1, grade 3) and was absent from 83. Pseudopyloric glands were absent in 85 specimens and were present in 12 (2, grade 1; 9, grade 2; 1, grade 3). Cysts were encountered in 10 specimens (8, grade 1; 2, grade 2) and were absent in 87 specimens. Only 2 specimens showed healed erosions (both grade 1).

Table V consolidates the findings in the antral mucosa. Thirty-three specimens (34 per cent) showed no change on either curvature. In 19 specimens (19.6 per cent) the changes were confined to the lesser curvature and, of these, the changes were focal in 15 and patchy in 4. In 2 specimens (2.1 per cent) there were isolated focal lesions on the greater curvature only. Ten specimens (10.3 per cent) showed changes over the whole lesser curvature with associated focal changes on the greater curvature. Of these, 2 showed only excess infiltrate. No specimens showed diffuse changes on the greater curvature alone. Both curvatures were similarly involved in 33 specimens (34 per cent). Of these, both curvatures showed focal lesions 12 times and small patchy lesions 9 times. The remaining 12 specimens showed diffuse changes: 4 showed excess infiltrate only, and 8 (8.2 per cent of entire series) showed diffuse parenchymal changes (chiefly atrophy and metaplasia). It is to be noted that 7 of these 8 specimens were among the 53 from persons over 50 years of age (an incidence of 13 per cent) and but one was among the 44 specimens from persons in the earlier decades (an incidence of 2.3 per cent).

Table VI consolidates the findings with reference to their distribution in the body mucosa. In 49 specimens (50.5 per cent) both curvatures were unchanged. Eight specimens (8.2 per cent) showed changes on the lesser curvature only. Three of these showed only excessive infiltrate, 3 showed focal lesions, and 2 showed patchy lesions. Seven specimens (7.2 per cent) showed changes on the greater curvature only. In one of these there was a moderate infiltrate only, while in 6 there were isolated focal lesions. In 3 specimens (3.1 per cent) there were diffuse changes on the lesser curvature (1 with excessive infiltrate only, 2 with parenchymal changes) and only focal lesions on the greater curvature. In one specimen (1 per cent) excessive infiltrate on the greater curvature was associated with focal changes on the lesser curvature. (In this case there was actually a similar infiltrate on the lesser curvature, but the presence of parenchymal change placed it in the distinctly abnormal category.) Twenty-nine specimens (29.9 per cent) showed comparable changes on both curvatures. Eight of these showed isolated focal or patchy lesions and 21 (7 with excessive infil-

TABLE V

Distribution of Changes in Antral Mucosa

Age group	Number of specimens	Normal on both curvatures	Abnormal on lesser curvature only				Abnormal on greater curvature only				Both curvatures abnormal							
				Parenchymal changes				Parenchymal changes			Changes throughout lesser, focal lesions greater		Changes throughout greater, focal lesions lesser		Both curvatures similarly involved			
																	Diffuse	
			Excess infiltrate only	Focal	Patchy	Through-out	Excess infiltrate only	Focal	Patchy	Through-out	Excess infiltrate only	Par-enchymal changes	Excess infiltrate only	Par-enchymal changes	Focal	Patchy	Excess infiltrate only	Par-enchymal changes
1–10	6	6																
11–20	4	1		1											1			1
21–30	11	6		2											1		2	
31–40	9	4		2	1						1					1		
41–50	14	5		3	1							1			2	2		
51–60	11	2		4				1				1				1	1	1
61–70	21	4		2	1							5			5	1		3
71–80	16	3		1	1			1			1	1			2	3	1	2
81–90	4	1													1	1		1
91–100	1	1																
Totals	97	33		15	4			2			2	8			12	9	4	8
First 5 decades	44	22		8	2						1	1			4	3	2	1
Second 5 decades	53	11		7	2			2			1	7			8	6	2	7

TABLE VI

Distribution of Changes in Body Mucosa

Age group	Number of specimens	Normal on both curvatures	Abnormal on lesser curvature only				Abnormal on greater curvature only				Both curvatures abnormal							
				Parenchymal changes				Parenchymal changes			Changes throughout lesser with focal changes greater		Changes throughout greater with focal changes lesser		Both curvatures similarly involved			
			Excess infiltrate only	Focal	Patchy	Throughout	Excess infiltrate only	Focal	Patchy	Throughout	Excess infiltrate only	Parenchymal changes	Excess infiltrate only	Parenchymal changes	Focal	Patchy	Excess infiltrate only	Diffuse parenchymal changes
1–10	6	6																
11–20	4	4																
21–30	11	7	1	1				1										1
31–40	9	6	1														1	1
41–50	14	9			1										1		2	1
51–60	11	6		1												1	1	2
61–70	21	5	1		1		1	2				1	1		2	2	1	4
71–80	16	6		1				2			1	1					2	3
81–90	4														2			2
91–100	1							1										
Total	97	49	3	3	2		1	6			1	2	1		5	3	7	14
First 5 decades	44	32	2	1	1			1							1		3	3
Second 5 decades	53	17	1	2	1		1	5			1	2	1		4	3	4	11

trate only) showed diffuse changes. Of the specimens with diffuse changes, 6 were from the 44 persons in the first 5 decades and 15 were from the 53 in the second 5 decades of life. Considering only the 14 specimens with parenchymal changes (14.4 per cent of the entire series), 3 were from the former group (an incidence of 6.8 per cent) and 11 were from the latter group (an incidence of 20.8 per cent).

TABLE VII

Summary of Findings in Antrum and Body Combined

Age	Males	Females	Total no. specimens	Antrum and body both normal	Body normal, antrum abnormal		Antrum normal, body abnormal		Both antrum and body abnormal			
					Focal, patchy, etc.	Diffuse	Focal, patchy, etc.	Diffuse	Focal, patchy, etc. in both	Diffuse in antrum, focal etc. in body	Focal etc. in antrum, diffuse in body	Diffuse both antrum and body
1–10	5	1	6	6								
11–20	3	1	4	1	2	1						
21–30	4	7	11	5	2		1		1	1		1
31–40	4	5	9	3	3		1				2	
41–50	8	6	14	5	4				2		3	
51–60	9	2	11	1	5			1	1	1	1	1
61–70	13	8	21	3	2		1		8	2	4	1
71–80	11	5	16	3	1	2			4	1	5	
81–90	2	2	4				1			1	2	
91–100	1	0	1				1					
Total	60	37	97	27	19	3	5	1	16	6	17	3
First 5 decades	24	20	44	20	11	1	2	0	3	1	5	1
Second 5 decades	36	17	53	7	8	2	3	1	13	5	12	2

The distribution of the changes for the whole stomach is shown in Table VII. Changes less than diffuse are here combined and the variations, from focal lesions to alterations over the whole of a single curvature, thereby lose identity. These specimens, however, are all quite sharply set apart from those with uniformly diffuse changes. Reference is made to the variations below.

Both antrum and body were free of change in 27 specimens (27.8 per cent). Twenty of these were from persons in the first 5 decades and 7 were from those in the second 5 decades.

Twenty-two specimens (22.7 per cent) showed changes in the antrum and a normal body mucosa. In 3 of this group the antrum showed diffuse changes (2 with parenchymal changes, one with excessive infil-

trate only). The remaining 19 showed less than diffuse changes and, of these, 17 showed focal or patchy lesions on one or both curvatures and 2 showed only excessive infiltrate on one curvature. As may be seen in Table VII, specimens in this group fall throughout the several decades and were proportionately somewhat more frequent in the earlier than in the later decades. In quantitative terms it is doubtful that any of the 19 should be considered significantly abnormal.

In 6 specimens (6.2 per cent) a normal antrum was associated with changes in the body mucosa. In 4 of these the body mucosa showed isolated focal lesions, one showed only an excessive infiltrate confined to the lesser curvature, and one showed diffuse parenchymal changes. In this group only one specimen, the last, can be considered quantitatively abnormal.

Both antrum and body showed changes of some degree in 42 specimens (43.3 per cent) and, as shown in Table VII, there was considerable variation in this group. In 16 of these the changes were less than diffuse in each of the two mucosal divisions. Seven of the 16 showed changes (2 with only excessive infiltrate) in the entire lesser curvature of antrum or body and the remainder showed focal or small patchy lesions. Six specimens showed diffuse changes in the antrum (one with excessive infiltrate only) and but focal lesions in the body. Seventeen specimens showed less than diffuse changes in the antrum and diffuse changes in the body. Here the body mucosa showed only excessive infiltrate 4 times and parenchymal changes 13 times. Twelve of the 17 were from persons over 50 years of age. In but 3 specimens were the changes diffuse in both antrum and body. One showed only excessive infiltrate in both regions, one showed excessive infiltrate in the antrum and parenchymal changes in the body, and one showed diffuse parenchymal changes in both.

Comment

Wide variation has been noted in the distribution of gastritic changes, ranging from focal to diffuse processes involving all of the antrum or body, but rarely both. There is no uniformity in the pattern of involvement of the antrum and body of the same stomach and these divisions must, in the main, be considered separately. These marked variations in distribution emphasize the necessity of designating the source of gastric material subjected to microscopic examination. It is clear that the presence of lesions on the lesser curvature of the antrum or body is not necessarily indicative of diffuse changes. On the other hand, extensive changes were encountered on the greater curvature only

as part of a diffuse process in the segment concerned. The uniformity of change from one area to another under these circumstances has been mentioned. It would appear, then, that changes, in excess of a focal lesion, encountered in a section of reasonable size from the greater curvature of the antrum or body well reflect the condition of the whole segment in so far as diffuse processes are concerned. The inference may be drawn, however, only in the case of stomachs otherwise free of disease.

While 27 specimens were free of change, many of those in which changes were found presented only isolated focal or patchy lesions. It seems quite certain that, if enough sections were prepared, few of the 27, at least among adults, would be entirely free of change. Consequently, a number of those tabulated as abnormal must be considered free of significant change. Where, short of diffuse involvement, to draw a line between normal and abnormal on the basis of quantitative change must be arbitrarily determined and remains uncertain. In this material there are no specimens which serve fully to bridge the gap between those showing focal or patchy lesions and those with diffuse processes. A somewhat intermediate position is taken by those specimens which showed changes along the entire lesser curvature with but focal or no changes on the greater curvature. This distribution was more frequently encountered in the antrum. How far on the anterior and posterior walls such a process may extend is not certain. The findings in this material suggest that, particularly in the body, the process is limited to the immediate vicinity of the curvature, but the number of pertinent cases is small and the impression could well be modified by a larger material. In those specimens which showed diffuse changes the process was quite uniform throughout and they were, consequently, sharply set apart from those with lesser degrees of abnormality. The uniformity of the process from one area to another suggests simultaneous involvement of the whole in its evolution. Here again the number of pertinent cases is too small to permit exclusion of spreading changes.

As in a previously reported series,[4] there is nothing in the available information concerning the patients represented which gives any clue to the origin or causes of the changes encountered. The only constant association seems to be that of advancing age. There is no necessity of believing that all of the changes encountered are causally related. That one may be observing qualitatively similar end-stages of reactions to a variety of causes seems likely. The striking separation between focal and diffuse processes as observed here may be a reflection of such differences.

It appears that the findings described are at least roughly indicative of the expected changes in the stomachs of individuals free of manifest gastric disease. The incidences recorded of the several degrees of change would no doubt be modified by a larger material. That the data fairly reflect the autopsy experience of this laboratory seems reasonable and is supported by collateral evidence. The 97 specimens on which this study was based were selected from a much larger group of similar specimens because of freedom from post-mortem change in the sections examined. It has been noted that those with diffuse changes presented a similar pattern throughout and, consequently, uniform changes in a relatively small area of the greater curvature fairly well reflect the condition of the whole in respect to the presence or absence of diffuse processes. Though unsuitable for inclusion in this series, those specimens which showed partial post-mortem changes in the rolls or those from which blocks only were available could be used for the determination of the incidence of diffuse changes. On this basis, diffuse gastritis has about the same frequency as noted above. Furthermore, allowing for the differences in technic of examination, the findings in this study compare rather favorably with those reported by me previously [4] on a similar material.

Summary

Gastritic lesions were found at autopsy in 70 (72 per cent) of 97 stomachs of persons free from manifest gastric disease. Of the 27 stomachs which showed no change, 20 were from the group of 44 persons less than 51 years of age and 7 were from the group of 53 persons over 50 years of age. There was no uniformity of involvement between antrum and body and the changes in each segment varied widely from isolated foci to diffuse alterations. Each segment is best considered separately. Many of the lesions encountered are not quantitatively significant but where, short of a diffuse gastritis, to draw a line between normal and abnormal on the basis of quantitative change is uncertain. The antrum was abnormal in some degree in 64 specimens (66 per cent of the total, 50 per cent of those from persons less than 51 years, 79 per cent of those from persons over 50 years of age). The lesions in the younger group were largely focal. Diffuse parenchymal changes were found in 8 specimens (8.2 per cent of the total), or in 1 (2.3 per cent) of those from persons under 51 years and in 7 (13 per cent) of those from persons over 50 years of age. The body mucosa was abnormal in some degree in 48 specimens (49.5 per cent of the total, 27 per cent of those from persons less than 51 years, 68 per cent of those from persons over 50 years of age). Diffuse parenchymal changes were found in 14 specimens (14.4 per cent of the total), occurring in 3 (6.8

per cent) from persons less than 51 years and in 11 (20.8 per cent) from persons over 50 years of age.

Conclusions

In otherwise normal stomachs, gastritic lesions are common. They predominate in the older decades, do not uniformly involve antrum and body, and within either area range from focal to diffuse in distribution.

Diffuse gastritis with parenchymal changes was found at autopsy in the antrum in 8 per cent and in the body in 14 per cent of a series of 97 stomachs of persons free of manifest gastric disease.

The gastritic changes observed in conjunction with other gastric lesions must be interpreted in the light of associated findings.

References

1. Hillenbrand, K. Histotopographische und histologische Untersuchungen über die sog. chronische Gastritis. *Beitr. z. path. Anat. u. z. allg. Path.*, 1930, **85**, 1–32.
2. Faber, K. Gastritis and Its Consequences. G. Forlagstrykkeri, Copenhagen, 1935.
3. Guiss, L. W., and Stewart, F. W. Chronic atrophic gastritis and cancer of the stomach. *Arch. Surg.*, 1943, **46**, 823–843.
4. Hebbel, R. Chronic gastritis. Its relation to gastric and duodenal ulcer and to gastric carcinoma. *Am. J. Path.*, 1943, **19**, 43–71.
5. Konjetzny, G. E. Die Entzündungen des Magens. In: Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie. Julius Springer, Berlin, 1928, **4**, Pt. 2, 768–1116.
6. Magnus, H. A. Observations on the presence of intestinal epithelium in the gastric mucosa. *J. Path. & Bact.*, 1937, **44**, 389–398.
7. Kalima, T. Pathologische-anatomische Studien über die Gastritis des Ulcusmagens nebst einigen Bemerkungen zur Pathogenese und pathologischen Anatomie des Magengeschwürs. *Arch. f. klin. Chir.*, 1924, **128**, 20–108.

Description of Plates

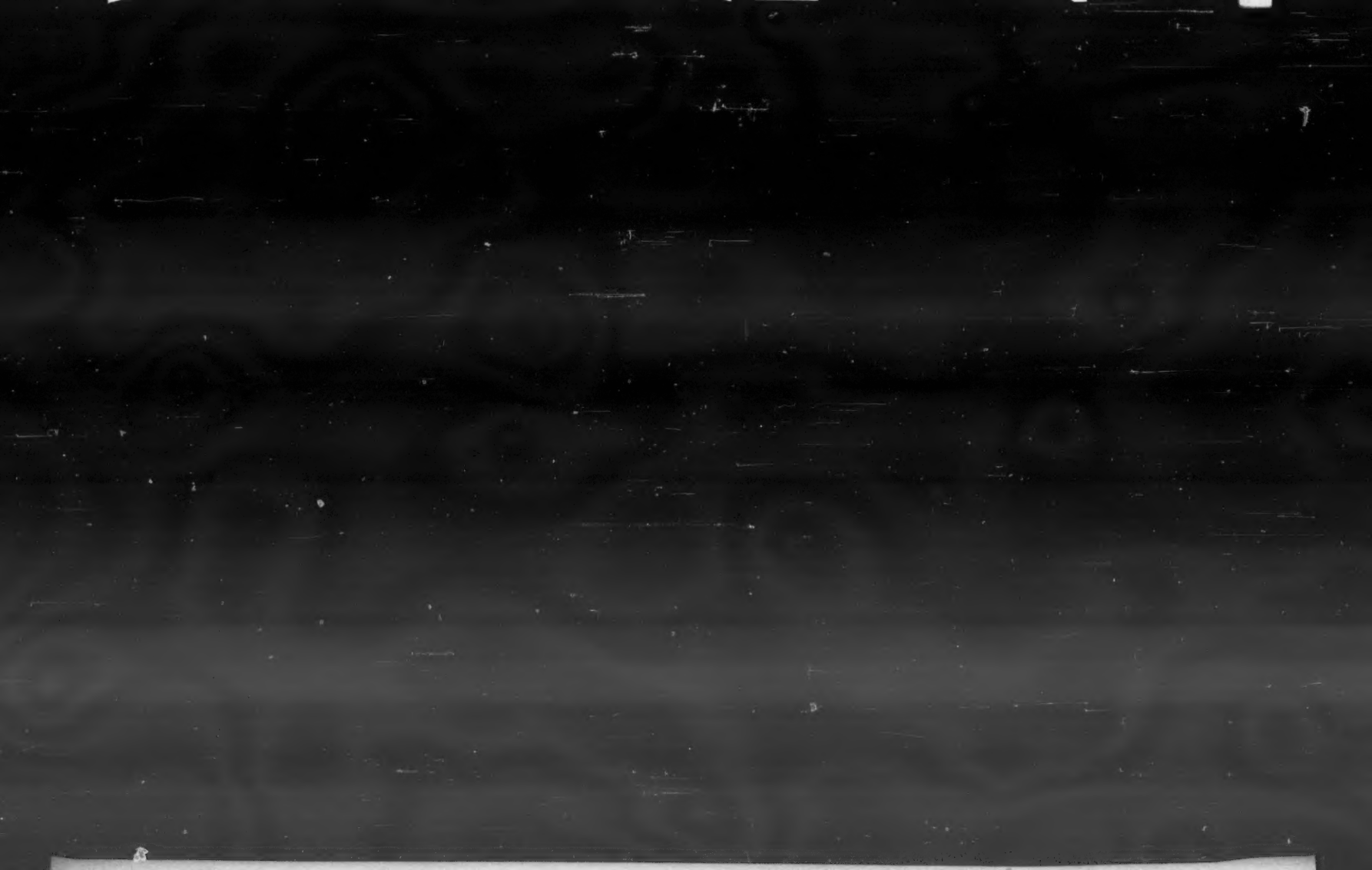
Plate 17

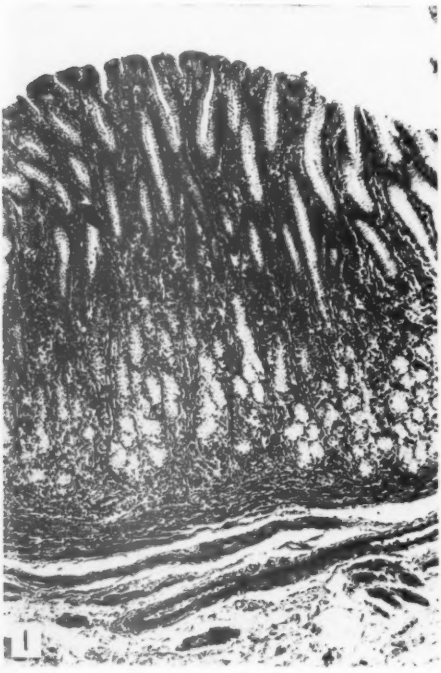
Fig. 1. Normal antral mucosa. Hematoxylin and eosin stain. × 65.

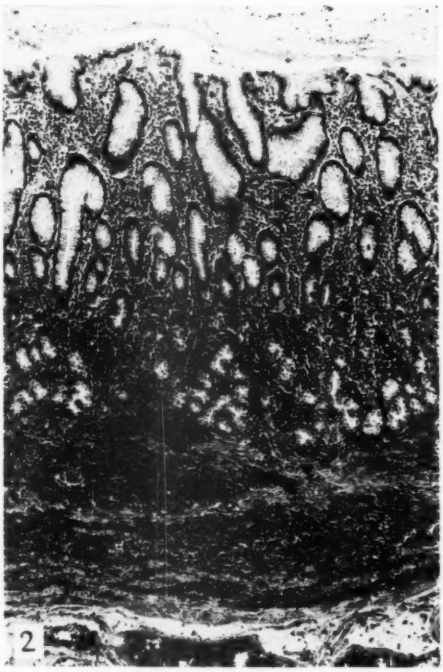
Fig. 2. Antral mucosa with moderately heavy lymphocytic infiltration which splits the muscularis mucosae. Hematoxylin and eosin stain. × 65.

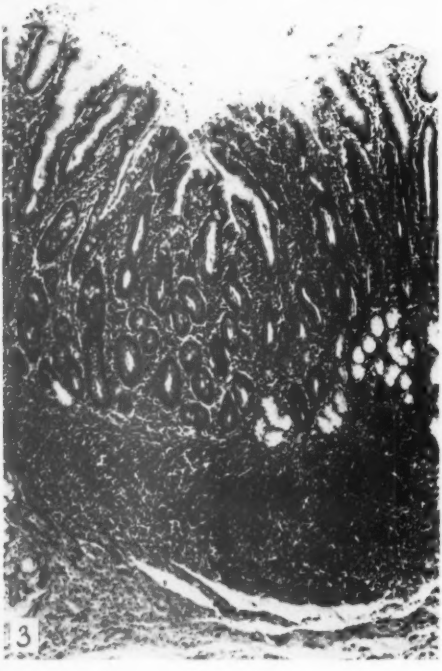
Fig. 3. Focus of intestinal metaplasia in antral mucosa adjacent to lymph follicle. Hematoxylin and eosin stain. × 65.

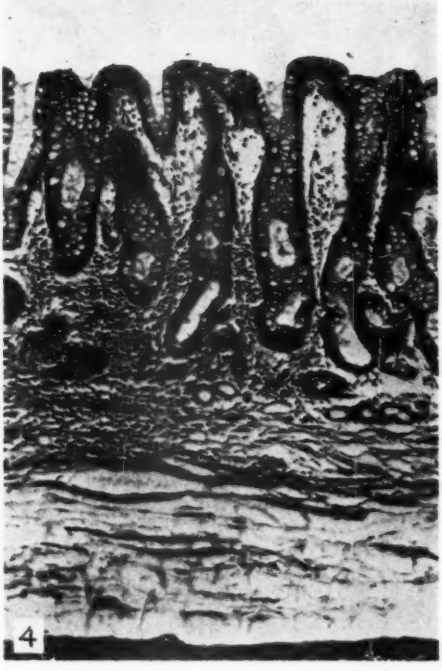
Fig. 4. Antral mucosa showing complete atrophy of glands and scant lymphocytic infiltration. Remaining crypts show epithelium of the intestinal type. Hematoxylin and eosin stain. × 65.











Hebbel Topography of Chronic Gastritis

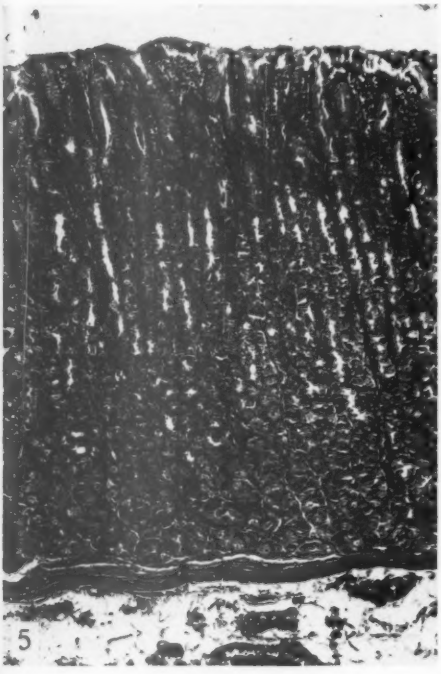
PLATE 18

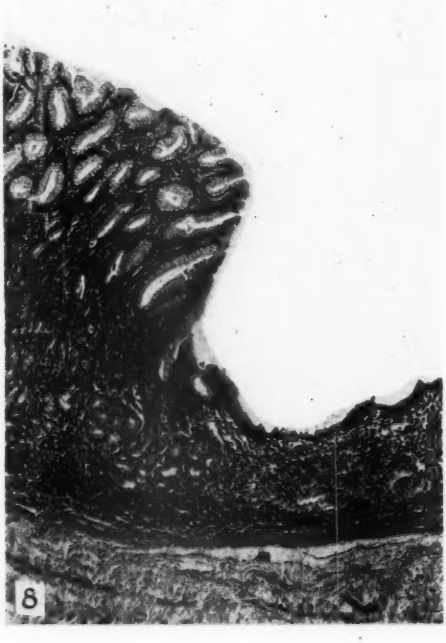
FIG. 5. Normal body mucosa with mild interfoveolar lymphocytic infiltration. Hematoxylin and eosin stain. × 65.

FIG. 6. Body mucosa showing heavy lymphocytic infiltration in superficial half and mild infiltration below. The crypts are deepened and the functional parts of the gland tubes correspondingly reduced. Hematoxylin and eosin stain. × 65.

FIG. 7. Body mucosa showing complete loss of normal glandular structure and presence of numerous pseudopyloric glands. Hematoxylin and eosin stain. × 65.

FIG. 8. Healed erosion in body mucosa. Adjacent glands partly replaced by pseudopyloric glands. Hematoxylin and eosin stain. × 65.





Hebbel Topography of Chronic Gastritis

DESTRUCTION OF CARTILAGE CELLS IN THE NEWBORN RAT BY BRIEF REFRIGERATION, WITH CONSEQUENT SKELETAL DEFORMITIES *

ROBERT O. SCOW, M.D.

(From the Division of Anatomy, University of California, Berkeley 4, California)

Following exposure to cold surfaces, remarkable distortion and retardation in growth of the tail and limbs of newborn rats have been observed (Scow, 1944). The deformities encountered took the form of short and wedge-shaped vertebrae producing marked angulation of the tail, bizarre curvature of the shaft, and rotation of the joint surfaces of the long bones, as well as shortening of the metatarsal, metacarpal, and phalangeal bones (Figs. 1 and 2). These phenomena followed the employment of refrigeration anesthesia in the course of which the rats were placed for 15 minutes in a glass beaker with the internal temperature reduced to $-5°$ to $-10°$ C. by partial submergence in a mixture of ice cubes and brine (Scow and Simpson, 1945). The resulting anesthesia, attended by temporary cessation of respiratory movements and marked reduction of cardiac rate, lasted from 8 to 15 minutes. Skeletal deformities were first noted several weeks later in about one-fifth of the 200 animals anesthetized. Further investigation demonstrated that deformities occurred only in those parts which had come in contact with the cold surfaces of the beaker, and were more severe following either a longer period of exposure or exposure to colder surfaces. These skeletal deformities usually were not associated with gross disturbances of general growth or of adjacent soft tissues, except in a few instances in which the exposure was sufficient to produce gangrene. The animals exhibiting skeletal deformities showed no other alterations in growth or differentiation.

The purpose of the following experiments was to determine the cause of the retardation in growth and differentiation affecting the skeletal elements. The tail of the newborn rat was employed because of its accessibility for exposure to cold, the ease with which the earlier stages of development of bone could be followed, and the readiness with which the deformities could be observed and recorded by roentgenograms.

EXPERIMENTAL PROCEDURE

A total of 55 newborn rats of both sexes of the Long-Evans strain were employed. After exposure to cold, the animals were returned to their mothers for nursing and were weaned on the 21st day of life. The parents did not hesitate to receive them. The mortality following experimental refrigeration was exceedingly small.

* Received for publication, December 31, 1947.

The walls of a glass beaker submerged in a mixture of ice cubes and brine are of a temperature sufficiently low (−10° C.) to produce deformities, but the time exposure is unduly long and the experiments difficult to control. It was found later that when the newborn rat was placed on the surface of a piece of ice sprinkled with salt, similar results could be obtained. The method proved difficult to standardize, however, and a period of 10 to 15 minutes was required to produce freezing of the tail.

The method adopted, therefore, was to place the tail of the 1-day-old rat in contact with the surface of the freezing plate of a freezing microtome. The plate was frosted "white" prior to the exposure and the tail was then gently held against it for 15 to 20 seconds until it became white. After exposure, the rat was placed in a moderately warm place (6 to 8 inches below a 40 watt electric lamp) for 1 hour before being returned to the mother.

The animals were sacrificed at varying intervals, from ½ hour to 38 days after exposure. In those permitted to survive for periods in excess of 10 days, the resulting deformities were observed and recorded by roentgenography. Calcification of the vertebral segments in rats younger than 10 days is inadequate to permit roentgenographic study.

For histologic study, the tail, skinned except for the distal 1 cm., was fixed in 10 per cent neutralized formalin, decalcified in 5 per cent nitric acid, and infiltrated and embedded in celloidin. The tissue was sectioned at 8 to 10 μ and the sections were stained with hematoxylin and eosin.

Observations

Macroscopic Study

The tail of the newborn rat * became white after being held for 15 to 20 seconds against the freezing plate. The "white stage" lasted 20 to 30 seconds and was followed almost immediately by a reddening of the whole tail which persisted for several hours. The hyperemia usually subsided by the twelfth hour, unless gangrene supervened, and was not accompanied by gross edema. Retardation of growth was first evident 3 to 4 days after freezing. Angulation and deformity of the tail usually were apparent after the fifth day. The deformity increased progressively as the animal became older. It is very important to note that, except in a few cases, there was no evidence of permanent damage to the soft tissue. In the older animals, the external appearance of the tail usually was indistinguishable from that of the normal, with the exception of the decrease in length or the angulation, or both. In those

* Average size of tail of these newborn rats: length, 1.5 to 1.8 cm.; thickness (at base of tail), 1.7 mm.

animals developing gangrene, the first evidence of its appearance was the maintenance of the hyperemia followed by a chocolate discoloration of the skin. The skin was then easily shed on the second or third day; in some instances, the end of the tail dried up and dropped off spontaneously on the eighth to tenth day.

Roentgenograms taken on the 10th day after freezing demonstrated irregular areas of failure of calcification in the vertebrae.* The calcified area of some of the affected vertebrae was wedge-shaped instead of being rectangular. In other vertebrae the calcified centra were shorter than expected, or had irregular shapes, or exhibited irregularities at their distal or proximal surfaces. The wedge-shaped vertebrae invariably were associated with angulation of the tail, which increased in degree and extent with age (Fig. 1). The epiphyseal line of the affected vertebrae in the 38-day-old animals was irregular in outline and in others the epiphyses occupied abnormal positions (Fig. 2).

Microscopic Study

The normal caudal vertebra of the newborn rat consists of a mass of hyaline cartilage, bounded by perichondrium peripherally and by an intervertebral disk (consisting of the nucleus pulposus encircled by the annulus fibrosus) on its distal and proximal surfaces (Fig. 3). The cartilage exhibits three general zones of development: (1) At either end, irregular isogenous groups of typical hyaline cartilage cells, succeeded by (2) a zone of flattened cells in columnar arrangement, and finally (3) a central area of hypertrophic or vesicular cells (which are undergoing or are soon to undergo calcification) (Maximow and Bloom, 1942). There is no endochondral or periosteal ossification and very little, if any, calcification of the cartilage at birth. The skin is firmly attached to the underlying vertebrae and attendant ligaments by means of a dense, fibrous network. The subcutaneous tissues of the tail, therefore, afford little opportunity for collection of fluids and development of edema.

On freezing, destruction of the cartilaginous vertebra varied in extent from a few scattered necrotic cells to necrosis of the entire cartilage (Fig. 4). The extent of damage seemed related to the depth of penetration of cold and could be determined readily by tracing the affected cells centrally from the surface to which cold was applied. Sometimes only the edge of the most lateral parts of the vertebra was damaged; at other times, the area of destruction included a greater portion of the vertebra. The large hypertrophic cartilage cells in the center of the

* The calcified area in the caudal vertebra of the normal 10-day-old rat consists of the calcified cartilage, the adjacent endochondral bone, and the periosteal bone. At this age, the epiphyseal plate and epiphysis have not been formed.

vertebra, which are the first to be replaced by bone, were the most resistant of all cartilage cells to the freezing process. The area of degeneration nearly always was defined by a curved border concave toward the injured side.

The changes in the cartilage cells, which were found to be permanent and associated with the death of the cells, were seen in the tail vertebrae 30 minutes after exposure to cold (the earliest specimens). The immediate changes consisted of basophilic condensation of the nucleus, loss of the nucleolus and filamentous structure, and eosinophilic granulation of the cytoplasm which was withdrawn from the lacunar wall, leaving a clear space. Four hours after exposure, the shrunken cytoplasm was still more eosinophilic and the nucleus more basophilic. The affected cells were found sparsely scattered throughout otherwise normal-appearing cartilage, or solely occupying large areas of the vertebrae. At later stages the cells showed further shrinkage and the nucleus accepted the eosinophilic stain more readily. In 30-day-old animals, not uncommonly, islands of these eosinophilic ghosts could be seen enclosed within the bone which had now developed. At no time had they initiated any foreign body reaction.

In order to demonstrate further that these alterations were brought about immediately by freezing, epiphyseal cartilage from the knee joints of several three-quarter-term bovine fetuses were studied. The material was obtained and used less than 1 hour after the death of the mother. Cellular changes similar to those found in the tails of rats after freezing were observed in the unfixed tissue immediately after sectioning small pieces of the epiphyseal cartilage with the freezing microtome. These necrotic changes also were observed in small pieces of the cartilage which were frozen for 1 minute, fixed in 10 per cent neutral formalin for 2 days and then sectioned with the freezing microtome. However, cartilage fixed for 2 days in formalin before freezing and sectioning did not show these changes.

The earliest signs of repair were seen 30 hours after freezing, when large areas of the cartilage containing very faint eosinophilic ghost cells were invaded from the periphery by cells of a fibroblastic nature (with basophilic cytoplasm and large basophilic nuclei containing nucleoli). The perichondrium and vertebral borders of the intervertebral disk appeared to be undergoing considerable activity, yet very few mitotic figures were seen. In some of the older specimens, islands of living cartilage cells appeared to have arisen from scattered, surviving cartilage cells (Fig. 7).

An interesting phenomenon was seen in the 20-day specimens in which the vertebrae were wedge-shaped. On the unfrozen side, the bone appeared to be almost normal (*e.g.*, bone with periosteal ossification on the lateral side, and endochondral ossification at each end; Fig. 7) and was separated from the frozen side of the vertebra by a narrow area about one quarter the length of the long side, composed of the "shrunken" necrotic cartilage cells. This necrotic area was attached at both ends to the adjacent nucleus pulposus. The nucleus pulposus was no longer enclosed by the annulus fibrosus and also had become displaced to the shorter side. On the short side of the vertebra—that exposed to freezing—bone, twice as wide and less than one-half as long as that on the unfrozen side, had arisen from the periosteum. No endochondral ossification could be observed on this short side. It would appear that the excessive periosteal bone formation was the result of either stimulation by cold or by the adjacent, destroyed cartilage cells.

The nucleus pulposus in several specimens was displaced laterally to the damaged side, occasionally escaping from the annulus fibrosus. The nucleus pulposus first appeared to move laterally as the uninjured side of the vertebra increased in length (Fig. 6).

One is struck by the selective damage to the tail caused by brief refrigeration, namely, destruction of cartilage with other tissues remaining relatively intact. In some instances, the cartilage cells of the entire vertebra were necrotic, yet there was little damage to the other structures of the tail. The next most susceptible tissue, less frequently injured, was the muscle lying adjacent to the lateral walls of the vertebra. In very few instances, the collagenous fibers of the subcutaneous tissue showed degenerative changes, and least frequently seen was necrosis of the peripheral layer of the epidermal cells. The blood vessels never were found to contain thrombi. The cells of the intervertebral disk (nucleus pulposus and annulus fibrosus), of the tendons of the tail, and of the perichondrium did not seem to be susceptible to cold with such brief exposure.

Discussion

Changes in Cartilage Cells

The great susceptibility of cartilage to the lethal effects of brief refrigeration is an interesting phenomenon and one which leads to remarkable disturbances of skeletal growth and development in the rapidly growing newborn rat.

Rischpler, in 1900, reported that all tissues which he studied suffered

under the influence of lowered temperatures even when the exposure was for a duration of but 3 to 4 minutes. The damage consisted primarily of an alteration of the cell and its nuclear structure, and it was directly proportional to the duration and degree of cooling. The location and structure of each cellular element also played a rôle in determining the degree of damage. He noted that the epiphyseal cartilage cells and their nuclei in the mouse tail showed signs of damage 20 minutes after freezing by an ether spray.

In extensive study of the effects of freezing on living tissues, Burckhardt, in 1926, reported findings similar to those discussed here. He examined the effect of freezing on the elbow joints of rats at varying ages to evaluate the relative sensitivity of the tissues involved, and to assess their relative regenerative powers. He found epiphyseal cartilage, bone, and articular cartilage of newborn rats to be most sensitive, and observed also the distinct line of demarcation between the injured and uninjured cells within the cartilage, as noted in the present observations. On the day following freezing, he found that the injured cartilage cells stained weakly and, later, disappeared, possessing no regenerative power. Articular cartilage of somewhat older animals, bone tissue (both compact and spongy) of all older groups, bone marrow, and periosteum were found to be next in degree of sensitivity. The developed cartilage of adult or nearly adult rats was considerably less sensitive. Relative to the large blood vessels and the skin, he could infer only that their function remained preserved even after the most severe freezing.

Strandell (1941) found that cartilage and, more specifically the unclosed epiphysis, was especially sensitive to cold.

Haas (1942) reported a microscopic study of the interaction between crystal violet and various human embryonal tissues which were sectioned at 15 to 20 μ on a freezing microtome. He described a "positive reaction" as consisting of a "microscopic interaction in the presence of nitrous acid between a particulate product derived from crystal violet and an unknown cytoplasmic component which is especially abundant in the region of the cytoplasmic membrane." He found this positive reaction to be specific for certain types of infantile cartilage. The subperichondral cells and cells in the central portion of epiphyseal cartilage gave a positive reaction whereas "the large swollen [cartilage] cells proximal to the deposits of osteoid tissue," the perichondrium, and the neighboring fibrocytes failed to react. All of the noncartilaginous tissues studied failed to give a positive reaction whereas the reac-

tion of adult cartilage was not uniform. It is interesting to note that Haas has found this reaction to be specific for epiphyseal cartilage with the exception of "the large swollen [cartilage] cells proximal to the deposits of osteoid tissue." The latter cells also were found in the rat tails in this study to possess a low sensitivity to freezing in contrast to the other cell types of young cartilage. This "immunity" might be related to some change in the vesicular cells during their degeneration, prior to invasion by the vascular connective tissue in the process of endochondral ossification.

In view of the high sensitivity of cartilage to cold, it is suggested that the "positive reaction" and its specificity for the cartilage cells, as described by Haas, may depend upon a chemical or physical change in some component of the protoplasm of cartilage cells brought about by freezing (on the microtome), during the preparation of specimens for sectioning, which is related to the death of the frozen cartilage cells as observed in this study. Observations of the reaction of cartilage to freezing have been infrequently noted in the literature. Many of the studies carried out on freezing have been made on pathologic specimens from human adults, or on portions of animals in which cartilage (especially epiphyseal or young cartilage active in endochondral ossification) was not present.

Skeletal Anomalies

The subsequent development of skeletal anomalies in the rat tails and limbs which had been exposed to brief freezing on the first day of life has been clearly shown to be the result of destruction of cartilage cells active in endochondral ossification. The degree of the deformity depends upon two main factors: (1) The initial extent of cartilage destruction (*e.g.*, a whole vertebra, or a portion); and (2) the duration of time after the freezing. Since cartilage is the precursor of bone, laying down a "mold" of the skeletal part and later taking active part in the linear growth of the bone at the epiphyseal lines, its destruction obviously will lead to deformities. As an example, in the vertebra of a newborn rat which has sustained damage to the cartilage in its lateral portion, growth at the ends of the diaphysis occurs with an increase in the length of the vertebra on the less damaged side, whereas the other side may partially or completely fail to increase in length because of the dead cartilage cells. The difference in the length of the two sides of the vertebra accompanied by a distortion of the pattern of the normal vertebra will increase progressively. An example is cited of the knee

joint of one rat which was held against the cold surface and sustained lethal damage to cartilage cells on that side of the adjoining two bones.* By the 38th day, the joint surfaces of these two bones had each revolved more than 100°, with an accompanying curvature of the bone shaft.

Löhr, in 1930, reported an isolated case of a 16-year-old child who had frozen one hand with subsequent development of deformity and lack of growth in the little finger, which roentgenographically showed an absence of the epiphyseal line in the distal phalanx. In the hands of 5 adults, similarly exposed, he was unable to find damage in or near the joints.

Burckhardt (1930) also noticed this difference in response to freezing by cartilage in animals of growing age and in those of adult age. He felt that epiphyseal cartilage was sensitive to acute and chronic freezing, and that the damage was irreparable. He produced interference with growth following applications of cold (CO_2) to the elbow of the rat, and observed that the lower end of the humerus (shaft and distal end) remained smaller than is normal. The dead cartilage of the epiphysis either underwent gradual disappearance or remained as a hyaline band.

Mechanism of Necrosis

It has been shown clearly that cartilage cells undergo immediate changes following exposure to cold below the freezing point of water and that these changes—basophilic condensation of the nucleus with loss of its nucleolus and filamentous structure, and an eosinophilic granular condensation of the cytoplasm with a withdrawal of the cytoplasm from the lacunar wall—are associated with the failure of these cells to take their normal active part in the process of endochondral ossification. It is believed that these cells therefore suffer an immediate death. Areas of living cells, which have been shown to take part in the growth of the tail, are seen directly in contact with areas of the affected cells, and the line of demarcation between the two areas is observed to be either straight or concave towards the affected or frozen side. This line probably represents the depth of penetration by the cold.

The mechanisms by which cold brings about tissue destruction can

* The epiphyseal lines of the tibia and the femur adjacent to the knee joint were each found to have rotated about 120 to 150° (in relation to the normal position of each) in a 38-day old rat after the knee joint had been held against the freezing microtome plate for 1 minute on the first day of life (Fig. 8). (The roentgenogram of this knee joint is seen in Fig. 2). On the first day of life, the epiphyseal body had not yet appeared according to the roentgenograms; thus the true epiphyseal line was not present. The latter was probably represented by the line of active endochondral ossification. Undoubtedly the epiphyseal line-to-be was injured on one side by freezing with a consequent reduction of increase in length of that side of the bone; and with a relatively uninterrupted growth on the opposite side, the epiphyseal line as well as the articular surface of the bone was rotated as compared to the normal knee joint.

be divided into two general types: *indirect* and *direct*. Much interest has been shown recently in the *indirect* effects of cold, by which a disturbance in the circulation to the part is brought about, with consequent degenerative processes which may lead to gangrene (Adami, 1910; Askanazy, 1913; Smith, Ritchie, and Dawson, 1916; Lake, 1917; Lewis, 1941; Greene, 1943; Friedman, 1945; Lange, Boyd, and Loewe, 1945; Kreyberg, 1946). This has been proposed as the pathogenesis of immersion foot, trench foot, and frostbite. In a large body of tissue, such as the extremities of man, the amount of the tissue damaged is in excess of that which has been penetrated by cold (Lake, 1917). In these cases, there is a latent period between exposure and loss of tissue.

Cold can affect the tissue, also, by its *direct* action upon individual cells (Askanazy, 1913; Smith *et al.*, 1916; Blackwood and Russell, 1943), as occurs in the superficial tissue destroyed by cold, and also in small bodies of tissue which are easily penetrated by cold. In studies of effects of cold upon living tissue, ranging from bacteria through larger plant cells to animal tissues, both normal and malignant, several different processes have been suggested: (1) Cellular rupture (Chandler and Hildreth, 1935); (2) alteration of enzyme systems (Haines, 1937; Safford and Nathanson, 1944); (3) alteration of proteins in protoplasm (Askanazy, 1913; Lake, 1917; Haines, 1937); (4) dehydration by salt disturbances (Rischpler, 1900; Lambert, 1912; Moran, 1929); and (5) acute aseptic inflammation (Brownrigg, 1945; Kreyberg, 1946).

The tail of the newborn rat is so small that penetration of a large portion of the exposed tissue by cold is possible. No evidence of vascular occlusion was seen in the tissues studied. The changes were seen immediately after freezing, much too soon for them to be due to vascular deficiency. They were noted also in portions of cartilage tissue completely removed from the organism as a whole and from the vascular system. Because of these observations, it is felt that the changes are the result of the direct effect of cold upon the tissue. It is difficult to evaluate the various mechanisms listed for the mode of action of the direct effect of cold, but it can be said that the microscopic observation of marked alteration in cytoplasm and nucleus suggests that the cellular retardation observed in this study is the result, at least in part, of an alteration of proteins of the cell.

Summary

Cartilage cells in the unossified caudal vertebrae and limbs of newborn rats were found to be highly susceptible to brief refrigeration. The other tissues studied and listed in order of decreasing susceptibility

to cold were muscles, subcutaneous tissue, and skin. The cells of the intervertebral disk, perichondrium, future periosteum, and caudal tendons did not seem susceptible. The effect of cold on osseous tissue was not studied because of the absence of bone in the tail of the newborn rat.

Progressive marked alteration and retardation of growth with bizarre deformities were observed in these skeletal parts, following very brief exposure to cold.

These skeletal changes have been shown to result from necrosis of the cartilage cells which are normally active in lengthening and shaping skeletal parts through endochondral ossification.

The findings presented support the hypothesis that the lethal effects of cold act directly upon these cells, and that the changes include a marked alteration of the proteins in the cytoplasm and nucleus. No thrombi were seen.

The intervertebral disk adjacent to injured caudal vertebrae was observed to be shifted laterally to the frozen side, apparently by the differential growth of the frozen and the unfrozen parts of the vertebra. The nucleus pulposus often escaped from within the annulus fibrosis in these specimens.

Grateful acknowledgment is made to Dr. J. B. deC. M. Saunders for his advice and generous assistance throughout this study.

BIBLIOGRAPHY

Adami, J. G. The Principles of Pathology. Lea & Febiger, Philadelphia & New York, 1910, 1, 287.

Askanazy, M. Aussere Krankheitsursachen. In: Aschoff, L. Pathologische Anatomie. G. Fischer, Jena, 1913, pp. 54–56.

Blackwood, W., and Russell, H. Experiments in the study of immersion foot. *Edinburgh M. J.*, 1943, 50, 385–398.

Brownrigg, G. M. Frostbite. *Am. J. Surg.*, 1945, 67, 370–381.

Burckhardt, H. Knochenregeneration. *Beitr. z. klin. Chir.*, 1926, 137, 63–147.

Burckhardt, H. Über Kälteschäden an den Knochen und Gelenken. *Zentralbl. f. Chir.*, 1930, 57, 1851–1854.

Chandler, W. H., and Hildreth, A. C. Evidence as to how freezing kills plant tissue. *Proc. Am. Soc. Hort. Sc.*, 1935, 33, 27–35.

Friedman, N. B. Pathology of trench foot. *Am. J. Path.*, 1945, 21, 387–433.

Greene, R. The immediate vascular changes in true frostbite. *J. Path. & Bact.*, 1943, 55, 259–267.

Haas, G. M. Studies of cartilage. III. A new histochemical reaction with high specificity for cartilage cells. *Arch. Path.*, 1942, 33, 174–181.

Haines, R. B. The effect of freezing on bacteria. *Proc. Roy. Soc., London, s. B.*, 1937–38, 124, 451–463.

Kreyberg, L. Tissue damage due to cold. *Lancet*, 1946, 1, 338–340.

Lake, N. C. An investigation into the effects of cold upon the body. *Lancet*, 1917, 2, 557–562.

Lambert, R. A. The effects of cold on animal tissues. *Proc. New York Path. Soc.*, 1912–13, **12**, 113–121.

Lange, K., Boyd, L. J., and Loewe, L. The functional pathology of frostbite and the prevention of gangrene in experimental animals and humans. *Science*, 1945, **102**, 151–152.

Lewis, T. Observations on some normal and injurious effects of cold upon the skin and underlying tissue. III. Frostbite. *Brit. M. J.*, 1941, **2**, 869–871.

Löhr, W. Die Verschiedenheit der Auswirkung gleichartiger bekannter Schäden auf den Knochen Jugendlicher und Erwachsener, gezeigt an Epiphysenstörungen nach Erfrierungen und bei der Hämophilie. *Zentralbl. f. Chir.*, 1930, **57**, 898–909.

Maximow, A. A., and Bloom, W. A Textbook of Histology. W. B. Saunders Co., Philadelphia & London, 1942, ed. 4, 138–141.

Moran, T. Critical temperature of freezing—living muscle. *Proc. Roy. Soc., London, s. B.*, 1929–30, **105**, 177–197.

Rischpler, A. Ueber die histologischen Veränderungen nach der Erfrierung. *Beitr. z. path. Anat. u. z. allg. Path.*, 1900, **28**, 541–592.

Safford, F. K., Jr., and Nathanson, M. B. Clinical observations on tissue temperatures. *Arch. Surg.*, 1944, **49**, 12–22.

Scow, R. O. Retarding effect of brief refrigeration upon skeletal development in the newborn rat. (Abstract.) *Anat. Rec.*, 1944, **88**, 457.

Scow, R. O., and Simpson, M. E. Thyroidectomy in the newborn rat. *Anat. Rec.*, 1945, **91**, 209–226.

Smith, J. L., Ritchie, J., and Dawson, J. Clinical and experimental observations on the pathology of trench frostbite. *J. Path. & Bact.*, 1915–16, **20**, 159–190.

Strandell, G. Om Köldskador. *Nord. med.* (*Hygiea*), 1941, **10**, 1077–1089. (Cited by Friedman, 1945).

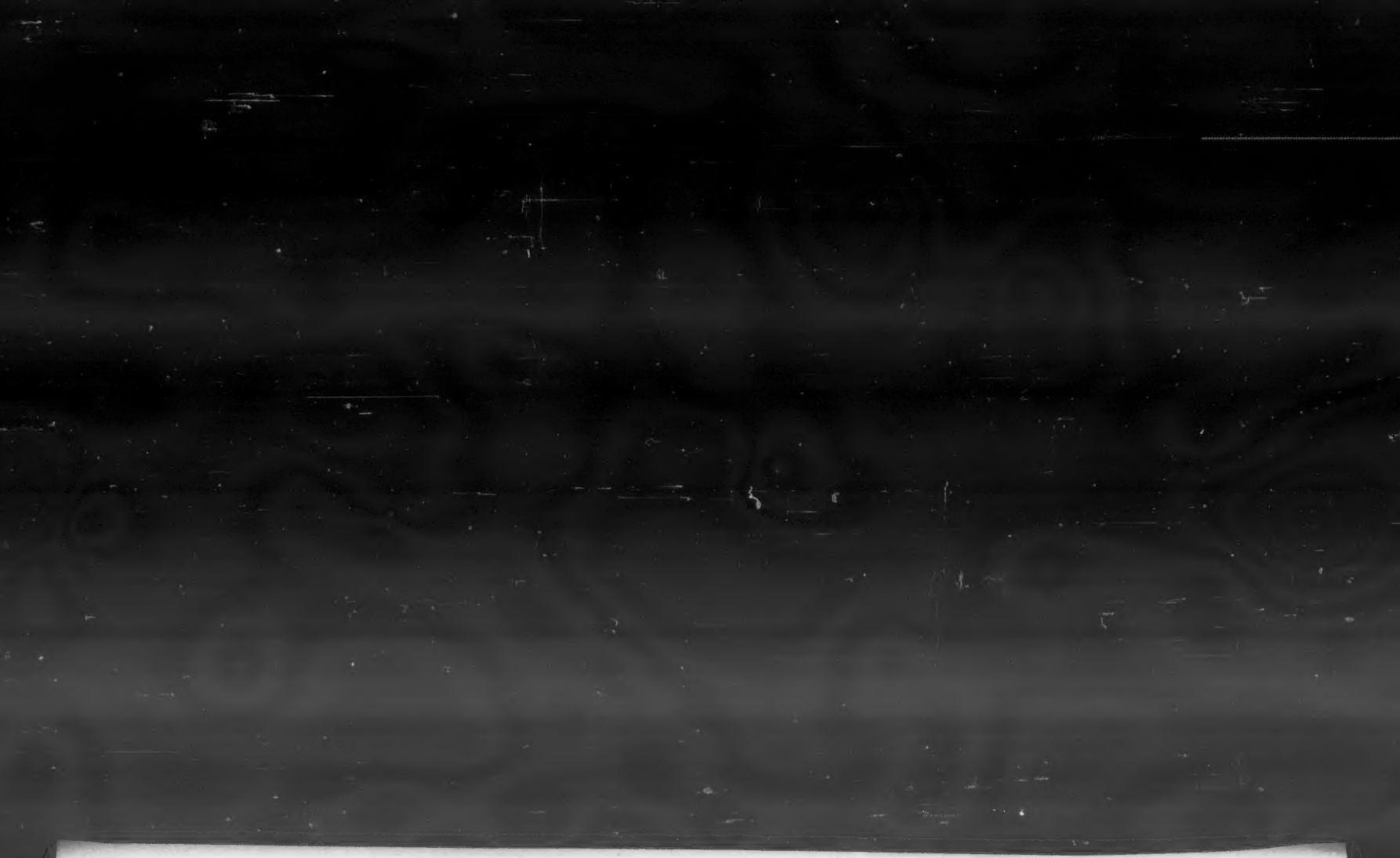
[*Illustrations follow*]

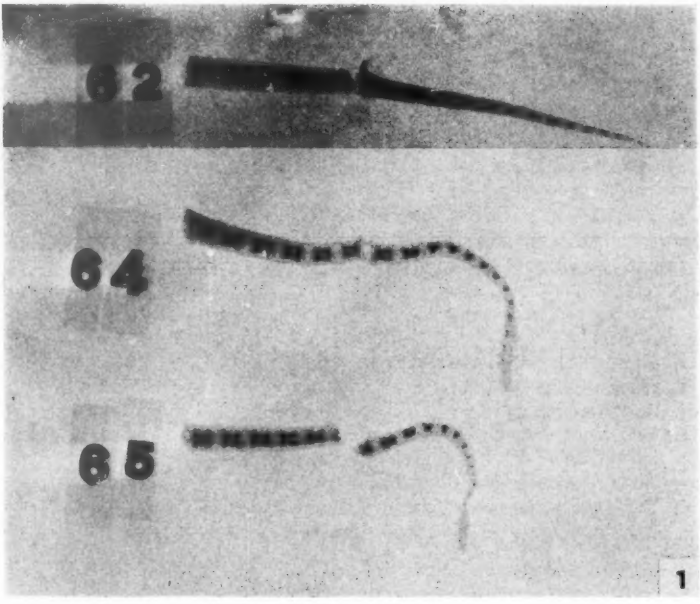
DESCRIPTION OF PLATES

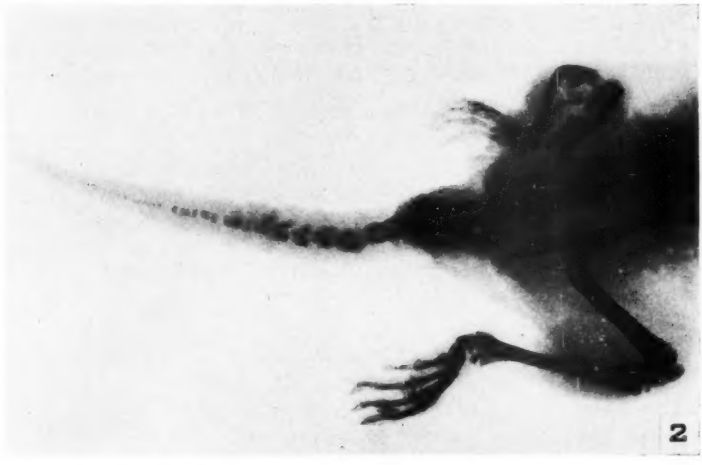
PLATE 19

FIG. 1. Rat 62. Roentgenogram of the tail of a 20-day-old normal rat. Rats 64 and 65. Roentgenogram of the tails 20 days after brief exposure of the tail to cold on the first day of life. Of note are the wedge-shaped vertebrae associated with the angulation of the tail. (See Fig. 7 for histologic section.) Natural size.

FIG. 2. Roentgenogram taken 38 days after the tail, left knee joint, and both feet were held against a cold surface for about 1 minute on the first day of life. Shortening and curvature of the shaft of the left femur and tibia adjacent to the knee joint are seen. The more distal caudal vertebrae also show evidence of damage by their shortness, irregular calcification, and asymmetry. (See Fig. 8 for histologic section of left knee joint, and text for discussion.) Natural size.







Scow Destruction of Cartilage Cells by Refrigeration

PLATE 20

FIG. 3. Longitudinal section of caudal vertebrae of a normal newborn rat. (There is a remnant of the notochord in the midline of the vertebrae connected to the nucleus pulposus of the intervertebral disk.) Hematoxylin and eosin stain. × 10.

The key to the numbers on the diagrammatic tracings of Figures 3 to 7 is as follows:

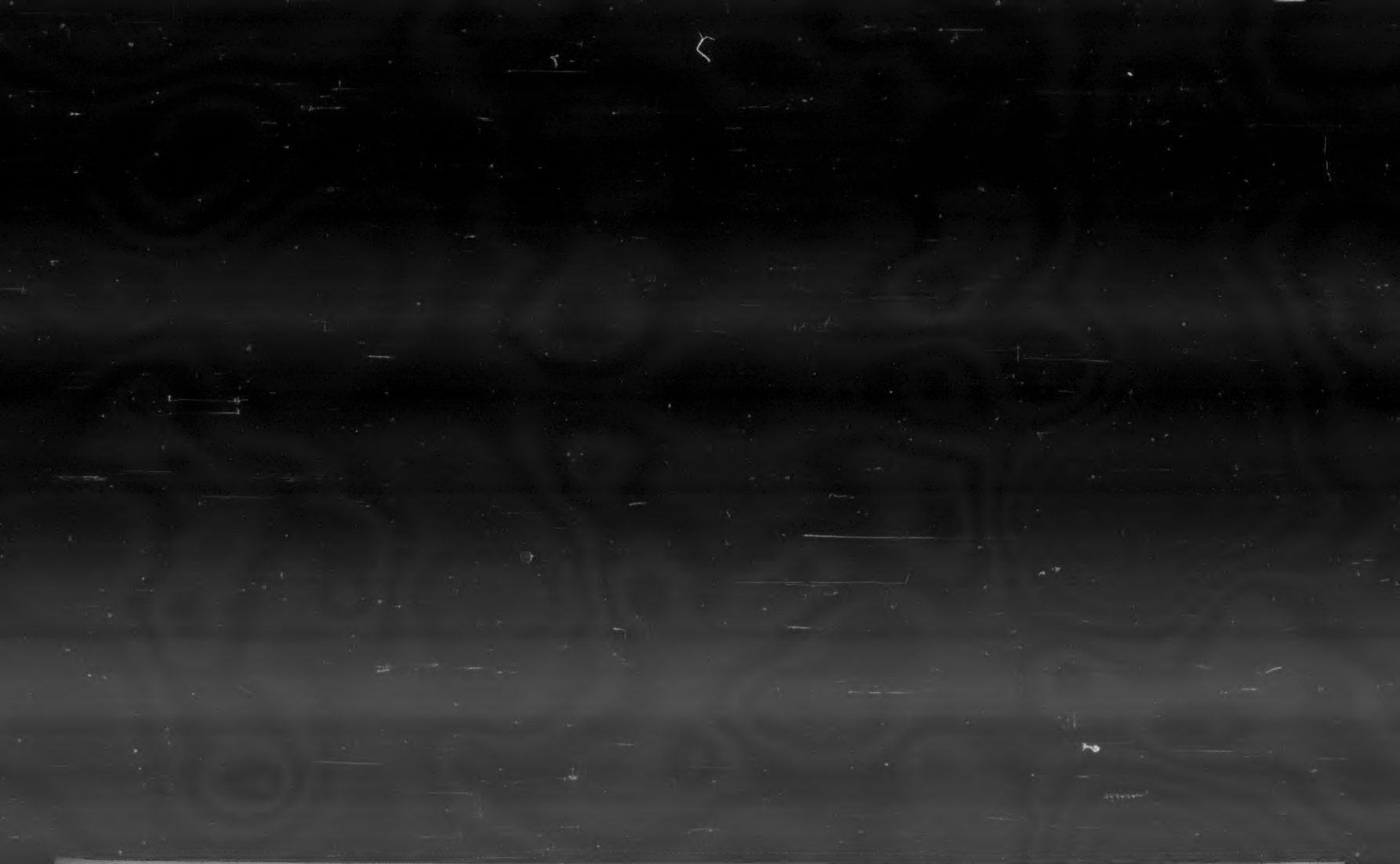
1. Normal, unossified caudal vertebra (cartilage)
2. Nucleus pulposus
3. Annulus fibrosus
4. Portion of unossified caudal vertebra (cartilage) which has been damaged by freezing
5. Bone developed by periosteal ossification
6. Cartilage taking active part in endochondral ossification
7. Bone developed by endochondral and periosteal ossification

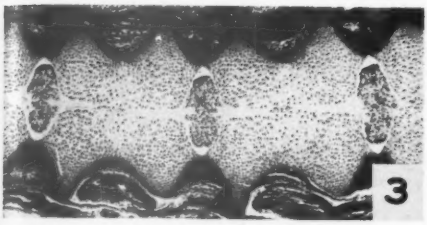
FIG. 4. Longitudinal section of the tail vertebrae of a newborn rat sacrificed ¾ hour after brief exposure of the tail to cold. The necrotic cells with shrunken nuclei and cytoplasm seen in area 4 may be compared with normal cartilage cells in area 1. Hematoxylin and eosin stain. × 10.

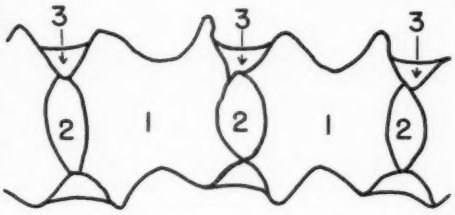
Tail vertebrae seen in Figures 4, 5, 6, and 7 were all similarly treated. The side of the tail seen in the lower portion of the photomicrograph was held gently for 10 seconds against an ice-cold surface (see text).

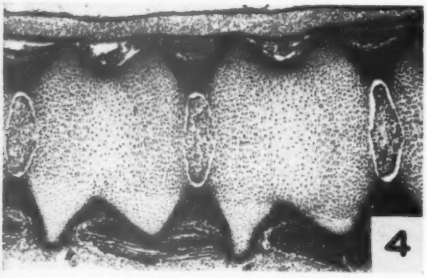
FIG. 5. Longitudinal section of caudal vertebrae of a rat sacrificed 2½ days after the tail was frozen on the first day of life. Hematoxylin and eosin stain. × 10.

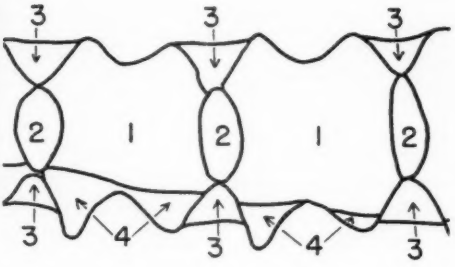
FIG. 6. Longitudinal section of caudal vertebrae of a rat sacrificed 4 days after brief freezing of the tail on the first day of life. Of note is the wedge-shaped vertebra with migration of nucleus pulposus (area 2) to the damaged side. Apparently normal development of vertebra may be observed on the uninjured side. Hematoxylin and eosin stain. × 10.

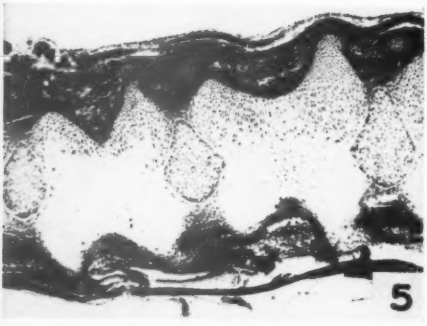


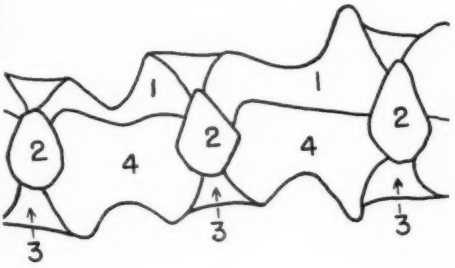


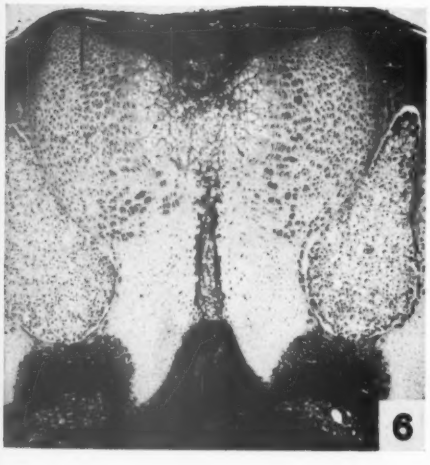


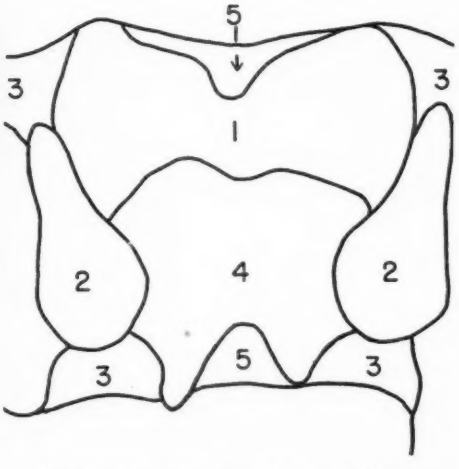










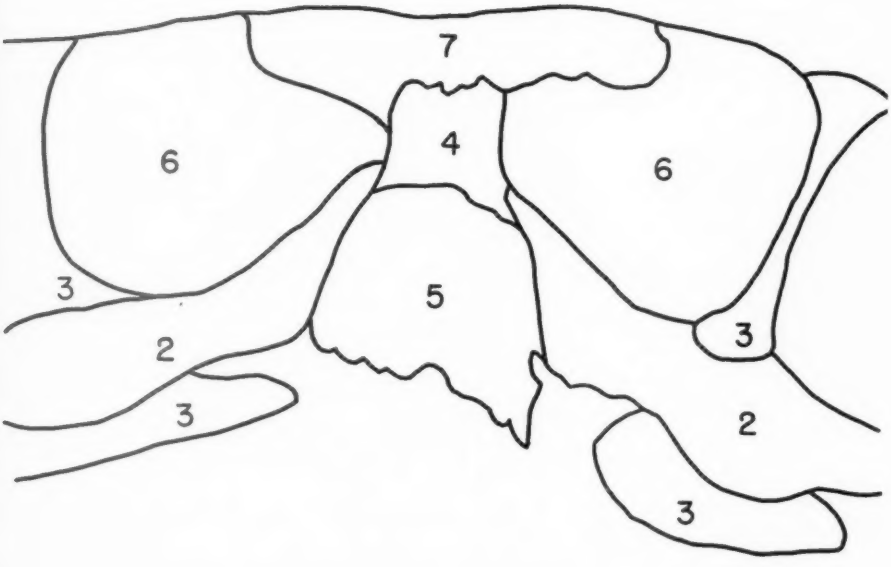


Scow

Destruction of Cartilage Cells by Refrigeration

PLATE 21

FIG. 7. Longitudinal section of a tail vertebra of a rat sacrificed 20 days after brief exposure of the tail on the first day of life. "Herniation" and migration of the nucleus pulposus (area 2) to the injured side may be observed, yet this area retains its connection with the remnant of injured cartilage (area 4). Of note is the difference between the length of the injured portion of the cartilage and that of the normally growing vertebra at the top of the photomicrograph (the uninjured side). Hematoxylin and eosin stain. × 10.



Scow

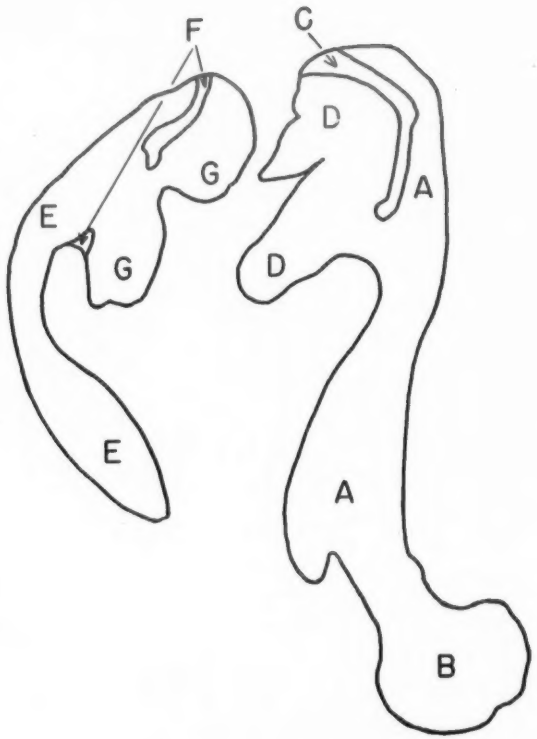
Destruction of Cartilage Cells by Refrigeration

PLATE 22

FIG. 8. Longitudinal section of the left femur and tibia of a 38-day-old rat, which were held against a cold surface for 1 minute on the first day of life. (See roentgenogram in Fig. 2.) Of note are the curvature of the shaft (A and E), interruption of epiphyseal lines (C and F) adjacent to knee joint, and rotation of articular surfaces of the two bones (see text for further description). Hematoxylin and eosin stain. × 3.5.

The key to lettered areas is as follows: Femur: A, shaft; B, head; C, distal epiphyseal line; and D, condyles. Tibia: E, shaft; F, proximal epiphyseal line; and G, condyles.

Scow



Destruction of Cartilage Cells by Refrigeration

EFFECT OF DIET DURING PREGNANCY UPON THE INCIDENCE OF CONGENITAL HEREDITARY DIAPHRAGMATIC HERNIA IN THE RAT

Failure to Produce Cystic Fibrosis of the Pancreas by Maternal Vitamin A Deficiency *

Dorothy H. Andersen, M.D.

(From the Departments of Pediatrics and Pathology, College of Physicians and Surgeons, Columbia University, New York 32, N.Y.)

The initial purpose of these experiments was to attempt to produce cystic fibrosis of the pancreas in newborn rats by means of breeding on a diet containing the minimum amount of vitamin A compatible with reproduction. The hypothesis that diet during pregnancy might play a rôle in the production of this lesion was based on three considerations which appeared valid when the experiments were begun in 1939. (1). Cystic fibrosis was known to occur in several siblings but no instance was known of its occurrence in more distant relatives.[1] Several such cases have since been observed by myself and others.[2] A disease of this pattern of incidence might be due to some untoward circumstance of pregnancy and is not necessarily of genetic origin. (2). The lesions found consist of obstruction of various epithelium-lined ducts, especially those of the pancreas. The obstruction is now believed to be the result of abnormalities in the material secreted.[3,4] (3). Obstruction of ducts by metaplastic epithelium had been described in experimental vitamin A deficiency.[5]

It seemed within the range of possibility and compatible with the facts then known, that the disease might be the result of vitamin A deficiency in the fetus. Since clinical vitamin A deficiency is not a characteristic of the mothers of patients with cystic fibrosis of the pancreas, a mechanism for the production of fetal deficiency without maternal deficiency must be postulated if this be the cause of the disease. Three explanations were devised: First, that an anomaly of maternal metabolism might lead to fetal deficiency; second, that the fetus, owing to a genetic abnormality, had an unusually high requirement of the vitamin; and third, that a degree of vitamin deficiency might exist which was adequate for the mother but inadequate for the fetus. Hence, either in the presence of a genetic metabolic anomaly or in normal animals, there might exist a level of vitamin A in the maternal diet which was compatible with maternal health and fetal deficiency.

The first step in testing this rather elaborate scheme was to demonstrate by animal experimentation that vitamin A deficiency during

* This investigation was supported by the Commonwealth Fund.
Received for publication, February 27, 1948.

fetal life leads to cystic fibrosis of the pancreas. The following experiments failed entirely in this objective. They have, however, provided convincing support of the more general hypothesis that a level of nutritional deficiency during pregnancy may occur in which the mothers show few or no signs of deficiency but the young die in the first day or two of life. As the experiment progressed it also became apparent that another important concept had been proved, namely, that the frequency of expression of a hereditary congenital malformation may be increased by deficiency in the maternal diet.

The literature on the production of congenital malformations by means of deficient diets during pregnancy has been reviewed recently by Warkany.[6] The malformations which have been described as the result of breeding on diets deficient in vitamin A include a variety of anomalies of the eye,[7-9] the teeth, [10] and malformations resulting from disproportionate growth of the skeleton and nervous system, including hydrocephalus [11] and blindness due to constriction of the optic nerve.[12] A preliminary report of the occurrence of diaphragmatic hernia in the young of vitamin A-deficient rats was made by me in 1941.[13] No data have been found relating to the effect of deficiency of specific nutritional substances during pregnancy on the incidence of malformations in the human being. Nonspecific nutritional deficiency leads to lowered fertility and increased infant mortality.[14-16]

Methods

Experimental Animals. The rats used in all but one experiment were of a colony which originated in 6 white rats which were purchased in 1929 and bred in the laboratory since that time without the addition of a new strain. Breeding has not been consistently by brother and sister matings. The rats used as breeders for the continuation of the colony have been selected on the basis of size, general condition, and freedom from infection of the first litters. Post-mortem examination of the lungs of all animals for evidence of infection has therefore been part of the routine of all experiments for the 8 years and over 3000 animals preceding the present study. This procedure has decreased the incidence of lung infections from about 50 per cent to something less than 10 per cent of the adult animals of the stock colony and has inadvertently provided evidence that diaphragmatic hernia of major degree was at least not common in the strain under routine breeding conditions. No instances of this anomaly were observed and if they had been common they could hardly have been overlooked.

The rats used in one experiment (no. VIII) were of the Long-Evans

strain. They were bred from 2 females and a male obtained in 1939. Breeding was not exclusively by brother-sister matings but care was taken to breed only within the strain. By chance the original rats were all black, although some of the young were hooded. Black rats were selected as breeders, so that the rats used in experiment VIII were black. This precaution was taken to minimize the chance of admixture with the white stock colony.

In order to ascertain fertility and also the incidence of resorption of the fetuses and the duration of pregnancy, all matings were carried out after a few days or weeks of examination of the vaginal smear. The rats were mated only when the smear was of the estrous type and they were examined on the following day for sperm or plug. After positive mating the male was removed from the cage and the female was weighed. She was weighed subsequently about twice a week. Infertility was nearly always the result of failure to have normal estrous cycles, the more deficient animals continuously showing cornified epithelium in the smears. Occasional resorptions were observed. The chief complication of pregnancy was delayed parturition, which has previously been reported in vitamin A-deficient rats.[17]

Diets. The stock diet used throughout was as follows:

Whole wheat flour	670 gm.
Casein	150 gm.
Dried milk	100 gm.
Calcium carbonate	15 gm.
Sodium chloride	10 gm.
Butter	50 gm.
Brewer's yeast	5 gm.
	1000 gm.

In this diet the main source of vitamin A was butter and the amount of the vitamin was somewhat variable. The batches of diet were made up weekly and kept in closed tins. This diet has been used routinely for the stock colony.

The vitamin A-deficient diet, diet 30, was as follows:

Casein, defatted	180 gm.
Cornstarch	570 gm.
Vegetable fat (crisco)	50 gm.
Wheat germ	50 gm.
Salt mixture	50 gm.
Brewer's yeast	100 gm.
Viosterol	15 drops
	1000 gm.

The casein was subjected to continuous extraction with hot 95 per cent ethyl alcohol for a total of 20 hours over a period of 3 days by the method described by Sperry.[18] After 3 to 6 hours of extraction the casein was removed from the extraction bag, ground, sieved, and replaced. After conclusion of the extraction it was dried in air, sieved, and stored in cans until use. In the preparation of the diet the dry ingredients were first mixed. The viosterol was worked thoroughly into the crisco and this was then worked into the dry materials. The diet was made up weekly. It was not assayed for vitamin content but this was apparently adequate except for vitamin A, since rats showed normal growth and reproduction for many generations when given diet 30 supplemented by vitamin A.

Haliver oil (Abbott) was used as the source of vitamin A. Various dilutions were made with Wesson oil, the calculations being based on the manufacturer's assay (1 gm. = 50,000 international units; 1 gm. = 40 drops; 1 drop = 1250 i.u.) The various dilutions used were given letters for convenience, with the following estimated content of vitamin A: X, 625 i.u. per drop; Z, 125; A, 25; B, 5; C, 1; D, 0.2; E, Wesson oil.

The supplement was given in amounts of 1 drop per day per rat and was fed three times a week by medicine dropper. Care was taken that only rats receiving the same supplement were kept in the same cage, but no attempt was made to prevent coprophagy.

Preliminary Experiments to Determine the Maximum Deficiency of Vitamin A Compatible with Reproduction

A series of experiments was carried out with small numbers of animals in order to determine the lowest dietary level of vitamin A compatible with fertility and the birth of young. In these experiments the factors to be considered were the age at which the rats were transferred from the stock diet to diet 30, the age at mating, the daily supplement of vitamin A, the length of time during which it was given, and the relation of this period to pregnancy and parturition.

Experiment I. At the age of 70 days, 12 female rats were transferred from the stock diet to diet 30. At 90 days they were divided into five groups and these were given supplements A, B, C, D, and E respectively. The rats were mated and all bore litters at ages between 113 and 142 days. Three litters were missing on the day after birth (on B, C, and D levels). The rest showed no abnormalities except for a lower average weight at weaning in the rats bred on the lower supplements. It was concluded that the rats were started on the diet too late to be adequately depleted of vitamin A.

Experiment II. Nineteen female rats were started on diet 30 at the age of 35 days and the supplements Z, A, B, C, and E were started at the age of 60 days, each level of supplement being given to 3 of 4 rats. Mating was begun at 80 days. Estrous cycles were irregular and fertility

was subnormal. However, 11 of the 12 rats receiving Z, A, or B levels became pregnant. None of the rats receiving C or E supplements were successfully mated. After positive mating the supplement was discontinued.

The 54 young of the 7 rats receiving the supplements A or Z appeared normal on the day of birth but disappeared one at a time during the first week, so that only 10 survived to the age of weaning (21 days). No external abnormalities were noted, and few of the young were autopsied since only fragments of them were found. Of the 4 rats on the B supplement, 2 died during parturition and the other 2 gave birth. One litter was born during the night and had been eaten by morning. The other litter was dead when found; *3 of its 7 members were found to have hernias of the right side of the diaphragm* and no abnormalities were found in the other 4.

From this experiment the conclusion was drawn that the initial depletion of vitamin stores was adequate and that the supplements at the Z, A, and B levels were sufficient to permit positive mating but that the total deficiency at the end of pregnancy in rats receiving B supplement was too great for survival of the young, and insufficient vitamin was stored at the A and Z levels of intake to provide for the demands of pregnancy and lactation when the vitamin was withheld after positive mating. The occurrence of one litter with 3 rats having diaphragmatic hernia was considered a chance finding.

Experiment III. It was apparent that the deficiency was not great enough for the production of abnormal young in experiment I and too great for normal parturition and survival of the young in experiment II. Experiment II was repeated, using the surviving young of the rats of Experiment I, to evaluate the rôle of the diet of the previous generation. The results were essentially similar to those of experiment II. The chief contribution of this experiment was the finding of *right diaphragmatic hernia in 3 of the 17 surviving young rats.*

Experiment IV. Experiment IV was designed to discover whether the neonatal death of the young in the previous experiments was due to inadequate lactation and also whether supplement given late in pregnancy would increase the proportion of young surviving. The breeding mothers were composed of three groups: *a.* One group was placed on diet 30 at 35 days and mating was begun at 70 days; supplement B was begun on the 60th day and continued through pregnancy. *b.* A second group was placed on diet 30 at 30 days and given no supplement until the 16th to 21st day after successful mating when they received an initial dose of 4 drops of "Z" followed by 2 drops on alter-

nate days. *c*. A third group used as controls was kept and bred on the stock diet. The plan was to breed one of the control rats on the day following each positive mating of one of the deficient animals, and exchange half of each litter at birth or the following day. The control young were marked by clipping the end of the fifth toe of the right fore-

TABLE I

Experiment IV. The Results of Exchanging Half of Each Litter of the Vitamin A-Deficient Rats with Half of Each Litter of Controls, to Determine the Rôle of Lactation in the Neonatal Mortality.

Experiment	Nursed by rat no.	Young of rat no.	No. of young	Mean wt., gm., at		
				1–3 days	14 days	21 days
IV–a	3618 Deficient	3618	3 (1 dead at 8 days)	6 (2 days)	17.5	24.5
		3601	4	7.25	18.25	21.75
	3601 Control	3618	2	6.0	26.5	37.5
		3601	5 (1 dead at 8 days)	8.8	27.75	39.5
IV–a	3639 Deficient	3639	4	5.25 (1 day)	Dead by	
		3644	4	5.25		
	3644 Control	3639	4	5.25	4th day	
		3644	4	5.25	26.25	44
IV–b	3669 Deficient	3669	3 (1 dead at 2 days)	6.0 (3 days)	17.0	32.0
		3660	4	7.5	18.75	36.0
	3660 Control	3669	3	7.3	25.0	44.3
		3660	4	9.0	27.0	49.0
IV–b	3696 Deficient	3696	1	Dead (2 days)		
		3661	4	10	28.5	49
	3661 Control	3696	3	Dead (2 days)		
		3661	4	10		45

foot. Each control mother therefore suckled half the litter of the experimental animal and half of her own litter, and each experimental mother likewise had a mixed litter. When necessary, young rats were discarded so as to have no more than 8 in each litter.

This plan contained too many hazards and was successfully executed in only 2 cases of the *a* and 2 of the *b* groups, a total of 4 successes in 14 rats started on the experiment (Table I). Of the remainder, one proved sterile, 2 resorbed their litters, success in obtaining parallel control litters was lacking in 2, and the remaining 5 litters were born dead.

In the four experiments in which the exchange of litters was safely made, the results were varied. In the first, both litters were weaned, with obviously better growth in the litter cared for by the control mother. In the second, the entire mixed litter cared for by the experi-

mental animal and also her young cared for by the control mother died within a few days. The results in the third pair of rats paralleled those of the first, and in the fourth all of the offspring of the experimental mother died by the second day while all those of the control mother did well, whichever mother cared for them. Thus an example of each possible result was observed. The answer to the question whether

TABLE II

Experiment IV. Incidence of Diaphragmatic Hernia in the Young of Rats Bred on Minimal Amounts of Vitamin A Compatible with Reproduction. The Rats Were Placed on Diet 30 at 35 Days of Age. Group a Received Supplement B from the 60th Day, Group b Received Supplement Z Beginning at the 16-19 Day of Pregnancy.

	Rat	Z given on day	Number of offspring			
			Born	Survived to 21st day	Autopsied	With D.H.
Group a	3618	—	7	4	7	1
	3639	—	8	0	1	0
	3641	—	4	0	3	1
	3621	—	7	0	7	2
	3626	—	6	0 (4 killed)	4	1
	3645	—	8	6	6	0
	3628	(resorption)				
Total			40	10	28	5
Group b	3669	16	7	6	5	0
	3696	18	7	0	3	1
	3654	19	5	0	5	5
	3658	19	9	0	9	9
	3694	18	12	0	12	0
	3664	Resorption				
	3665	No successful mating				
Total			40	6	34	15
Grand Total			80	16	62	20

neonatal death was due to deficient lactation was not decisive, but observation of the young led to the belief that the majority of the young of the deficient mothers were too feeble to suckle.

Although the objective of this experiment was not attained, in the course of the careful watch necessary to carry out the experiment, for the first time a large percentage of the young were rescued from maternal ingestion. In the course of post-mortem examination of the young of the experimental animals an important observation was made. *Hernia of the diaphragm was found in a total of 20 of the 61 animals examined,* most of them from litters of the *b* group in which supplement was withheld until late in pregnancy (Table II). All of the young of 2 litters, numbering 5 and 9 respectively, showed a hernia of the diaphragm of severe degree.

Experiments Directed at Diaphragmatic Hernia

Experiment V. The first move in experiment V was to attempt to reproduce the lesions observed in experiment IV-*b*. This and subsequent experiments were based on the following standard procedure. The rats were taken from the stock colony and placed on diet 30 at the age of 35 to 42 days. Beginning at about the 70th day a vaginal smear was examined each day. When the smear was of the estrous type the rat was left overnight with a normal male which had been bred on an adequate diet. On the following morning a vaginal smear was examined for sperm or a plug. The male was then removed. The female was weighed on this day and twice a week thereafter. On the 16th to 18th day following the day on which the male was placed in the cage, the female was weighed and if there had been enough gain in weight to indicate pregnancy, the rat was given 4 or more drops of Z supplement by medicine dropper (500 i.u. of vitamin A) and 2 drops every 2 days thereafter. When mating was unsuccessful and vaginal smears showed a continuous production of cornified epithelium without estrus, 2 drops of Z supplement sometimes were given, and this often sufficed to produce an estrous cycle and positive mating.

In experiment V, 14 rats were thus treated, together with 14 controls which also were placed on diet 30 but received Z supplement regularly from the 60th day of age and an additional 12 drops late in pregnancy between the 17th and 20th day. Four of the deficient rats failed to become pregnant and were found to have pulmonary infection. One positive mating was followed by a resorption. Nine litters were born, totaling 69 rats. Two entire litters and members of 5 others were found dead on the day of birth, a total of 22 rats. A careful watch was kept so that all rats except 2 were recovered before maternal ingestion. All living young were killed at once and all 67 of the recovered rats were autopsied (Table V). Twelve rats of 4 litters showed a diaphragmatic hernia. Eleven of the control rats gave birth to litters, totaling 78 young; of these, 4 rats, one from each of 4 litters, had hernias. The malformation could therefore be reproduced in a fair proportion of the young bred on the standard regime, but it occurred also in the control litters.

Experiment VI. In experiment VI further proof that a deficiency of vitamin A increased the incidence of the malformation was sought by several methods. The first of these was the breeding of 5 successive generations of rats on diet 30 with supplement Z, to determine the incidence of hernia and also to test severely the adequacy of the diet. The sixth generation was divided into 2 groups, one of which was placed

on diet 30 + E and the other, composed of littermate controls, on diet 30 + Z, the supplements being started at 60 days of age. Facilities did not permit the maintenance and breeding of all rats of 6 generations. F_1 consisted of 8 females and 4 males picked at random from the stock colony; therefore the experiment was not based strictly on inbreeding. In subsequent generations, brother and sister matings were frequently but not uniformly made; breeding was always with the animals of the

TABLE III

Experiment VI. The Incidence of Diaphragmatic Hernia in the Young of Rats Bred for Five Generations on Diet 30 + Z as Compared with the Sixth Generation, Half of Which Were Bred on Diet 30 with Supplement and Half Without. All but One of the F_6 Experimental Rats Had Ceased to Have Estrous Cycles and a Single Estrus Was Induced by One or More Doses of Two Drops of Z (250 i.u. of Vitamin A).

Generation	Bred on diet	Number of litters		Number of rats	
		Total	With D.H.	Total	With D.H.
F_0	Stock			8	1
F_1	30 + Z	8	2	35	2
F_2	30 + Z	7	1	34	2
F_3	30 + Z	9	2	60	2
F_4	30 + Z	9	3	51	6
F_5	30 + Z	9	1	35	2
F_6C	30 + Z	14	1	108	1
Total	Diet 30 + Z	56	10	323	15
F_6 Exp.	Diet 30	14	5	92	17

same generation for this experiment. In the first 5 generations a number of young were saved for breeding and the remainder killed. All were ultimately autopsied. Of a total of 415 rats, 14 were found to have a diaphragmatic hernia (Table III). In the sixth generation the females of 8 litters were distributed into 2 groups of 15 rats each, dividing the littermates as evenly as possible. There were 12 pairs of littermates. Fourteen rats of each group produced litters. The young were killed on the day of birth to prevent destruction of the evidence. Of the 92 rats in the experimental litters, 17 had hernias of the diaphragm, while one of the 108 rats in the control litters had this malformation (Table III). Therefore, it was apparent that, although the appearance of hernia was suppressed in large part for successive generations on an adequate diet, it reappeared in a generation given a deficient diet. The adequacy of diet 30 + Z supplement is attested by the high fertility and good growth and appearance of the rats maintained on it for 6 generations.

Experiment VII. In experiment VII, wherever possible, the rats

which had produced a number of young with hernias when bred on a deficient diet were given supplement Z three times a week at levels of 7 drops weekly for several weeks, while diet 30 was continued. They were then bred again, if possible to the same males, and the litters examined for hernias. In no instance did these second litters include a rat with a hernia (Table IV). The tendency to herniation was apparently suppressed in the presence of high dietary levels of vitamin A.

TABLE IV

Experiment VII. Comparison of the Incidence of Diaphragmatic Hernia in the Litters of Rats Maintained on a Deficient Diet with Subsequent Litters of the Same Rats after Vitamin Supplements Had Been Given.

First litter, diet 30; no supplement until late in pregnancy						Second litter, diet 30 with Z supplement					
Female	Male	Age	No. of young	No. for post-mortem	No. with D.H.	Z supplement from age	Male	Age	No. of young	No. for post-mortem	No. with D.H.
		days				*days*		*days*			
3654	3598	99	5	5	5	95	3598	136	2	1	0
3658	3597	99	9	9	9	94	3597	135	11	4	0
4413	4174	147	9	9	6	142	4157	188	9	9	0
4424	4174	142	6	6	1	141	4162	199	8	8	0
4429	4132	144	6	6	1	143	4162	184	9	8	0
4442	4117	104	11	11	8	100	4157	190	9	8	0
4297	3946	135	2	2	1	128	3953	238	3	3	0
4307	4112	130	7	7	3	124	4024	231	5	5	0
4333	4226	94	6	6	2	89	4226	158	9	9	0
Total 9 litters			61	61	36	9 litters			65	55	0

Comparison of the incidence of hernias among autopsied rats in first and second litters gives $X^2 = 51.3$, $P = <.01$.

Experiment VIII. In experiment VIII, in order to determine whether the appearance of hernias under these dietary conditions was a characteristic confined to our inbred strain of stock rats, experiment VI-*b* was twice repeated, using inbred rats of the Long-Evans strain. These rats were black, having been selectively inbred in this regard for the purpose of making sure that there was no accidental admixture with the white stock colony. The first batch was run in April and May and the second in September and October, 1941. The data are presented in Table V. One hernia was found in the young of one of the autumn experimental group and none were found in the controls. There is thus a marked difference in these two strains of rats in the tendency for diaphragmatic hernia to appear under the influence of vitamin A deficiency.

Experiment IX. An attempt was made in experiment IX to discover the genetic characteristics of the malformation, by producing a sub-

strain in which the incidence of hernias was uniformly high under experimental conditions. For this purpose female rats nos. 3654 and 3658 were chosen (Table VI). These rats had been part of experiment IV-*b* and had produced 5 and 9 young, respectively, all of which had

TABLE V

Comparison of the Incidence of Diaphragmatic Hernia in the Stock, D.H., and Long-Evans Strains on Diet 30 with and without Z Supplement.

Strain	Experiment	Experiment or control	Rats	Litters	No. autopsied	No. with D.H.	Percentage with D.H.	X^2 and P
Stock	V	Experiment	14	9	67	12		
		Control	14	11	78	4		
	VI F_6	Experiment	15	14	92	17		
		Control	15	14	108	1		
	Total	Experiment	29	23	159	29	18.2	$X^2 = 23.4$
		Control	29	25	186	5	2.7	$P = <.01$
D.H.		Experiment	9	7	34	14		
		Control	8	8	46	5		
	Carotene	Experiment	12	8	55	16		
	experiment	Control	12	11	84	5		
	Total	Experiment	21	15	89	30	33.7	$X^2 = 23.2$
		Control	20	19	130	10	7.7	$P = <.01$
Long-Evans	Spring	Experiment	12	10	68	0		
		Control	13	13	87	0		
	Fall	Experiment	11	8	43	1		
		Control	11	11	66	0		
	Total	Experiment	23	18	111	1	0.9	
		Control	24	24	153	0	0	$P = <.01$

Comparison of the stock and D.H. strains on the experimental regime gives $X^2 = 7.53$, $P = <.01$; and of these strains on the control diet comparison gives $X^2 = 5.5$, $P = <.02$. The infrequency of hernia in the Long-Evans strain is apparent.

had right diaphragmatic hernias, 13 of them complete. These rats had been littermates from the stock colony and had been placed on diet 30 at 36 days of age. They were mated to males nos. 3598 and 3597, respectively, which were littermates. All 4 of these rats were without hernias. After their participation in experiment IV-*b*, the females were continued on diet 30 and given 7 drops of Z weekly and partook in experiment VII, producing small litters in which no hernias were found. They were then both placed on stock diet and after several weeks were each bred twice more, all 4 litters being sired by rat no. 3598. These 3 rats, 3654, 3658, and 3598, are considered as the F_1 generation of the D.H. strain. The resulting strain produced a larger number of members with hernias than the original stock strain, but the incidence still varied with the diet. With the use of this D.H. strain some infor-

mation as to genetic pattern of the malformation was obtained (Text-Fig. 1 and Table VII). On the stock diet in a total of 328 rats, 107 (or 32.6 per cent) were found to have a hernia. The sex distribution was approximately equal, with 50 of 164 males and 57 of 156 females affected; the sex was not recorded in 8 unaffected rats. Of these hernias,

TABLE VI

Breeding Record of Rats 3654 and 3658, of Experiment IV. The D.H. Strain Was Derived from Litters 3 and 4.

Female	Litter	Male	Diet	Supplement	Parturition		No. born	No. for post-mortem	No. with D.H.
					Age	Day of pregnancy			
					days				
3654	1	3598	30	4 Z at 19th day	99	24	5	5	5
	2	3598	30	7 Z per week	136	24	2	1	0
	3	3598	Stock	0	217	24	9	7	1
	4	3598	Stock	0	308	?	4	4	1
3658	1	3597	30	2 Z on 19th day	99	25	9	9	9
	2	3597	30	7 Z per week	135	23	11	4	0
	3	3598	Stock	0	219	23	10	10	3
	4	3598	Stock	0	295	23	9	9	4

34 were pericardial, 13 were right incomplete, and 3 were right complete in the males; 49 were pericardial, 6 were right incomplete, and 2 were right complete in the females, a sex difference which is probably not significant.

The incidence of hernias in the young is significantly greater when both parents are affected than when neither is affected, while the inci-

TABLE VII

Experiment IX. Incidence of Hernias in the Young of the D.H. Strain in Relation to the Presence of Hernia in the Parents. Stock Diet.

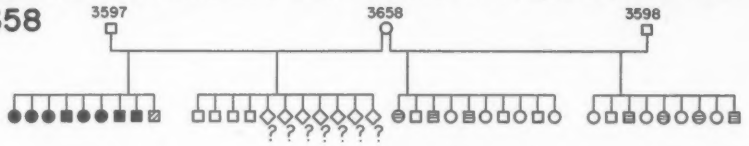
Hernia in parents		No. of litters	Young						Total		Percentage with D.H.
			Normal			Peric. D.H.		Other D.H.			
m.	f.		m.	?	f.	m.	f.	m.	f.	Rats	D.H.	
None	None	19	50	4	37	7	12	3	3	116	25	21.6
Peric.	None	14	16	4	19	8	9	1	5	85	27	31.8
None	Peric.	4	10		9	3	1	0	0			
Peric.	Peric.	16	27		25	8	17	8	0	85	33	38.8
?	None	5	11		9	8	10	4	0	42	22	52.4
Total		58	114	8	99	34	49	16	8	328	107	32.6

Comparison of incidence of hernia in young when both parents are affected in contrast to neither parent affected gives $X^2 = 7.1$, $P = <.01$. Comparison of litters of one versus no affected parent gives $X^2 = 2.6$, $P = <.10$. The former is significant, the latter is not.

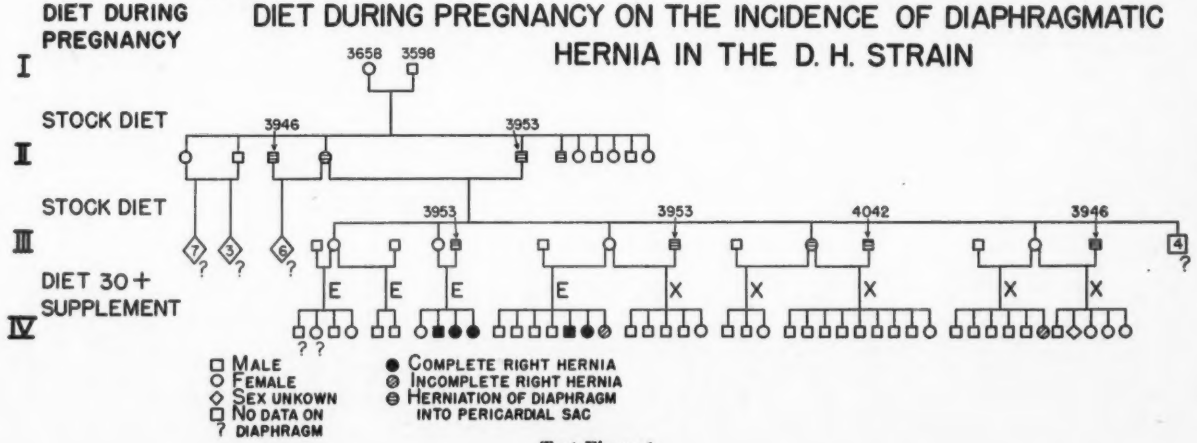
Peric. = pericardial hernia.

dence when only one parent is affected is intermediate between these two. The genetic pattern is therefore neither recessive nor dominant

THE EFFECT OF DIET DURING PREGNANCY ON THE INCIDENCE OF DIAPHRAGMATIC HERNIA AS SHOWN BY FOUR SUCCESSIVE LITTERS OF RAT #3658



PEDIGREE OF THIRD LITTER OF RAT # 3658 ILLUSTRATING EFFECT OF DIET DURING PREGNANCY ON THE INCIDENCE OF DIAPHRAGMATIC HERNIA IN THE D. H. STRAIN



Text-Figure 1.

and is not that of a sex-linked character. The malformation appears to be either the result of a general tendency within the strain or dependent on a number of genes.

Experiment X. The dietary factor which suppresses the expression of the hernia has been assumed to be lack of vitamin A, because no other nutritional substance is known to be present in effective quantities in the small amounts of haliver oil which were used. At the time that these experiments were performed, crystalline vitamin A was not available. Beta carotene therefore was used as a source of vitamin A

TABLE VIII

Experiment X. The Incidence of Diaphragmatic Hernia in the Young of Rats of the D.H. Strain Given Diet 30 with Supplements E, Z, or Carotene.

Supplement	No. of litters	Rats			Type of D.H.			
		No. D.H.	With D.H.	Percentage with D.H.	Peric.	Rt. compl.	Rt. inc.	Left inc.
Carotene	10	63	11	17.5	0	2	8	1
30 + Z	11	79	6	7.6	1	0	5	
30 + E	8	39	16	41.0	0	4	12	

Comparison of the Groups

Supplement	X^2	P
E vs. Z	12.4	<.01
E vs. B carotene	3.9	<.05
Z vs. B carotene	2.5	>.10

in an experiment designed to settle this point (experiment X). Rats of the D.H. strain were used, and were divided into 3 groups. They were subjected to the standard experimental procedure, starting the diet and supplements on the 30th day and mating on the 80th day. The supplements used were E, Z, and beta carotene dissolved in Wesson oil. The carotene solution contained 3 mg. of carotene per cc., and on the basis of 0.6 γ of carotene being taken as the equivalent of 1 i.u. of vitamin A, this solution had a theoretical potency equal to that of the Z supplement. In the ensuing experiment, hernias were found in 41 per cent of the young of the rats receiving E, in 7.6 per cent of those receiving Z, and in 17.4 per cent of those receiving carotene (Table VIII). The difference between the incidence of hernias in the Z and E groups is statistically significant but the difference between the carotene and E groups is less striking.

As the experiment progressed it became apparent that carotene was not as effective as Z in suppressing the appearance of hernias, and it seemed possible that the difference lay in the utilization of the two substances. The livers of the remaining rats were assayed for their content of carotene and vitamin A; although the data are few, they sup-

port the hypothesis that carotene was poorly utilized (Table IX). The results of this experiment support, but do not prove, the hypothesis that the effective agent is vitamin A. It is to be regretted that it was not possible to repeat this experiment with crystalline vitamin A.

TABLE IX

Experiment X. Assays of the Liver for Carotene and Vitamin A. Diet 30 with Supplements E, Z, or Carotene.

Rat no.	Hernia		Supplement	Liver					Young
	m.	f.		Weight	Carotene	Vitamin A	Carotene	Vitamin A	
				gm.	*γ per gm.*	*i.u. per gm.*	*γ per liver*	*i.u. per liver*	
4842	None	None	E	9.8	0.45	9.3	4.4	91	Missing
4906	Peric.	Peric.	E	9.1	0.27	25.8	2.5	234	Missing
4913	?	Peric.	E	6.6	0.40	3.2	2.6	21	No litter
4835	Peric.	None	Carot.	8.6	0.95	26.1	8.1	228	7 normal, 1 rt. compl.
4836	?	None	Carot.	7.5	1.19	22.3	8.9	167	1 normal, 3?
4843	Peric.	Peric.	Carot.	6.8	0.36	37.6	2.6	264	5 normal, 1 left inc.
4881	None	None	Carot.	9.8	0.42	16.1	4.1	158	9 normal
4891	None	None	Carot.	6.0	0.55	25.4	3.3	152	5 normal, 1 rt. compl., 2 rt. inc.
4897	Peric.	Peric.	Carot.	9.3	0.33	21.8	3.0	202	Missing
4903	None	Peric.	Carot.	7.7	0.41	27.4	3.2	211	Missing
4927	None	None	Carot.	9.9	0.39	19.4	3.8	192	12 normal, 2 rt. inc.
4839	Compl.	None	Z	7.8	0.80	1767	5.9	13,783	3 normal
4847	Peric.	Peric.	Z	7.8	0.29	1463	2.2	11,411	8 normal, 1 rt. inc.
4879	?	Peric.	Z	7.1	0.60	1050	4.3	7,459	9 normal
4880	None	None	Z	10.1	0.41	853	4.1	8,614	5 normal, 1 peric., 3 rt. inc.
4892	Peric.	None	Z	7.6	0.50	987	3.8	7,498	6 normal
4904	Peric.	Peric.	Z	8.0	0.54	915	4.3	7,316	4 normal, 1 rt. inc.
4909	None	Peric.	Z	7.6	0.47	1189	3.5	9,033	10 normal
4921	None	None	Z	6.6	0.50	919	3.3	6,062	Missing

Pathologic Anatomy

The Diaphragm. The hernias of the diaphragm were classified with respect to location and type as follows: (a) Right complete, with communication between the right pleural and peritoneal cavities; (b) right incomplete, consisting of a bulge of intact diaphragm into the right pleural cavity; (c) pericardial, consisting of a bulge of the diaphragm into the pericardial cavity; (d) left incomplete. No herniation through any natural opening such as the esophageal hiatus was observed. A complete defect was found only in the right leaf. A more detailed description of the malformations follows.

(a) A right complete hernia consisted of a defect varying from one involving the posterior half of the diaphragm to one in which the right leaf of the diaphragm was lacking (Fig. 1). In some instances a narrow shelf of muscle along the anterior diaphragmatic attachment remained. In all cases part or all of the right lobe of the liver lay in the right pleural cavity, and behind it, also in the pleural cavity, there were varying amounts of small intestine and the hepatic flexure of the colon. The right lung was small and was pushed up to occupy the upper part of the cavity. The right kidney usually was in its normal position. In one instance the right horn of a pregnant uterus was incarcerated in the right pleural cavity and was the cause of death. In another instance the mass of intestines in the right pleural cavity bulged through the mediastinum behind the pericardium but anterior to the esophagus to lie partly to the left of the spine, separated by a thin membrane from the left pleural cavity.

(b) The right incomplete hernia was found always in the posterior half of the diaphragm, and consisted of a roughly hemispheric sac consisting of pleura and peritoneum without interposed muscle and containing a nubbin of liver tissue which had grown to fill it (Fig. 2). The upper surface sometimes was flattened in the larger hernias. The size varied from one measuring 10 by 10 by 5 mm., involving about one-third of the right leaf, to small protuberances about 1 mm. in diameter. The majority were between 3 and 6 mm. in diameter. Occasionally several of these small bulges were present. The location varied but was always in the posterior half of the right leaf.

(c) The pericardial hernias were similar to the smaller right incomplete hernias except that they usually were more flattened and not always round (Fig. 3). They varied from 0.5 to 7 mm. in diameter, were sometimes multiple, and always contained a nubbin of liver.

(d) The left incomplete hernias were found infrequently, lay in the posterior half of the left leaf, and usually contained the upper half of

the stomach and occasionally a loop of gut, seen through the thin membrane.

The rats having a complete hernia died in the first day or two of life with few exceptions. The incomplete hernias were commonly found in rats bred according to the standard procedure which survived to adult life, and this lesion appeared to have no effect on their health or growth. Only a small percentage of the instances of neonatal death could be attributed to a complete hernia, however, and the cause of death usually was not apparent on gross post-mortem examination.

A comparison of the frequency of hernias of the various types in the young of deficient as compared with control mothers shows a greatly increased number of right complete hernias and a somewhat greater number of right incomplete hernias in the experimental animals, but no significant difference in the pericardial and left diaphragmatic hernias. The following figures summarize the incidence of the various types in all experiments of the stock strain, but do not include the rats of the D.H. or Long-Evans strains.

Type	*Experimental rats*		*Control rats*	
	No.	Percentage	No.	Percentage
Right complete hernia	32	50.8	4	16.7
Right incomplete hernia	28	44.5	12	50.0
Pericardial hernia	2	3.2	7	29.2
Left incomplete hernia	1	1.6	1	4.1
Total	63	100.0	24	100.0

Since many of these rats were observed in earlier experiments, it is probable that many of the pericardial hernias were missed, which may account for the lower incidence of this type in the stock rats as compared with the D.H. strain.

The hypothesis which most reasonably explains the morphogenesis of these hernias is a delayed growth of the diaphragm in the deficient animals. The posterior portion of the diaphragm is the last to close. Normally, the completion of the closure of the diaphragm precedes the return of the gut from the extra-embryonic celom of the umbilical cord. If the differential growth rates of the diaphragm and of the gut were altered so that the gut returned from the cord and increased in size prior to the closure of the diaphragm, it is reasonable to suppose that some abdominal organs might pass through the defect and prevent its closure.

Pancreas and Trachea. In the foregoing experiments it was possible to compare the microscopic changes in the trachea and in the pancreas

in rats bred on the deficient diet and examined at the age of 1 to 3 days with those in rats similarly bred but killed at weaning, in survivors continued on deficient diets, and in controls paralleling each group. It was possible to obtain sections of the organs of only a small number of rats from each experiment. These sections were stained with hematoxylin and eosin.

Of the rats bred of deficient mothers and examined at the age of 1 to 3 days, there were 8 from experiment III, 5 from experiment IV-*a*, and 8 from experiment IV-*b*, a total of 21. None showed any abnormality of the pancreas in the microscopic examination, when compared with 5 young from the stock diet controls. The trachea was examined in 4 rats of experiment IV-*a* and 7 of IV-*b*. Two of the latter showed early squamous metaplasia of the tracheal epithelium but the remaining 9 appeared normal. These 2 rats were among the 6 so examined in which a right diaphragmatic hernia was found.

Some of the rats of experiment IV in which the litters were successfully exchanged were examined microscopically, giving an opportunity to observe the effects of lactation by a deficient mother. No abnormalities of the pancreas were found in the 21 rats examined at the age of 21 to 23 days. Of these, 3 were bred and reared by deficient mothers, 4 were bred by deficient mothers and nursed by normal ones, 10 were bred by normal mothers and nursed by deficient ones, while 4 were normal controls. Squamous metaplasia of the tracheal epithelium was found in 5 of 20 rats examined, all 5 of which had been nursed by deficient mothers, but 4 were bred by normal mothers. Tracheitis was found in 9 more, 7 of which were nursed by deficient mothers. None of the rats bred and reared to weaning on a deficient diet were without either infection or metaplasia of the epithelium, while the 7 with normal tracheas were variously distributed among the other groups. Squamous metaplasia of the renal epithelium was found in only one rat, bred and reared on a deficient diet.

In addition to the above rats bred on deficient diets, there was also a large group from experiment III in which the pancreas was examined, at ages of 1 to 4 months. In none of these was there any suggestion of cystic fibrosis of the pancreas, although the acinar cells were small and atrophic in a few emaciated animals, and a few metaplastic cells were found in the pancreatic ductal epithelium in a few deficient animals. These observations were made on 12 animals bred on A supplement, 9 on B, 6 on C, 6 on D, and 8 on E supplement. In a total of 71 rats bred on levels of supplement leading to high neonatal mortality and an increased percentage of hernia (B, C, D, or E supplements), no pancreas showed changes suggestive of fibrocystic disease.

Metastatic Calcification. A large number of rats which had received diet 30 for a period of 10 weeks or more showed metastatic calcification. Of 44 rats which were killed before the age of 90 days and which had received diet 30 with various supplements, only 3 showed calcium deposits in the kidney, whereas 30 of 54 rats killed after the age of 90 days showed calcification in the renal parenchyma. The majority of these were killed at about 4 months of age. Rats on the stock diet rarely showed this change, and as the calcification was found in rats receiving low (B, C, D, and E) as well as high levels of supplement it was attributed to the diet rather than to hypervitaminosis D. It was found in rats bred on the stock diet and transferred to diet 30 before the 6th week as well as those bred on diet 30. In some of the rats showing calcium deposition in the renal cortex there was also calcification of the media of small arteries in the liver and elsewhere, with morphologic features suggestive of early Mönckeberg's sclerosis. There was no evidence of association of calcification with the hernias.

Cause of Death in the Neonatal Period. Except for the relatively small number of rats with a complete right diaphragmatic hernia, there was no gross pathologic change which explained the high neonatal mortality of the young of deficient animals. Microscopic sections of the lungs, liver, kidneys, spleen, and muscle gave no explanation of death. The majority of the deficient animals which survived to weaning and beyond had diffuse chronic bronchitis and interstitial pneumonia, but these changes were not found in the first few days of life. Observation of the living newborn rats led to the conclusion that the animals were too weak to breathe or to suckle, but they responded to painful stimuli. Whether the weakness was due to deficient function of muscle or of the nervous system was not determined.

Discussion

This series of experiments provides one more example of failure to attain the initial objective of an experiment with an incidental discovery of greater interest than the one originally sought. The general concept that congenital malformations may be produced by means of nutritional deficiency during pregnancy has been established previously. A variety of congenital malformations have been reported as the result of vitamin A deficiency by a number of authors working with various species of animals. It has been difficult to understand why the malformations reported by these workers varied so widely, especially in respect to conditions such as hydrocephalus and cyclopia, which are apparent without the aid of special technics. The present experiments suggest that species and strain differences may account for this.

In rats of the stock and D.H. strains fed on the stock diet, hernias of the diaphragm occurred in an appreciable number of cases. The incidence was increased to about 19 per cent in the stock strain bred on the deficient diet and was reduced to 2.7 per cent by means of generous supplements. In rats of the Long-Evans strain none of the control series and only one on the deficient diet was found to have a hernia. The strain differences are of a degree to be statistically significant. Nutritional deficiency therefore enhanced the degree of manifestations of this hereditary defect. The most plausible hypothesis is that in the affected strain the closure of the diaphragm was late in the strain generally, and that additional delay in the deficient animals led to an increased incidence of hernias.

Hernia of the type found in these rats is occasionally observed in the human subject but is less common than hernias through one of the natural openings of the diaphragm. It would be rash to infer that the deductions made from these experiments are directly applicable to the diaphragmatic hernia of man.

Two general concepts have arisen from these experiments which may prove to have application in the study of human neonatal mortality. The first is that a degree of specific nutritional deficiency during pregnancy may exist which is insufficient to produce obvious ill health in the mothers but which leads to death of the majority of the young; the cause of death may not be apparent on careful gross and microscopic pathologic examination. The second is that a tendency to a congenital defect may be carried as a genetic trait which is infrequently expressed under good nutritional conditions, but is frequently expressed when the diet during pregnancy lacks the appropriate specific nutritional factor. If congenital defects occur in this manner in man, it would be difficult to obtain evidence of the relationship of a specific malformation to a specific deficiency. Under general adverse nutritional circumstances in a population, however, one would expect a rise in neonatal mortality and in the incidence of congenital malformations.

Summary and Conclusions

A method has been devised whereby rats may be bred on a regime approximating the maximal deficiency of vitamin A compatible with reproduction. On this regime the majority of the young die during the first 2 days of life, while the mothers show few or no signs of deficiency.

No explanation of this high infant mortality has been obtained by gross examination in most cases, and microscopic examination of the lungs, liver, kidneys, pancreas, and striated muscle also failed to explain

death. No changes suggestive of cystic fibrosis of the pancreas were found.

Rats of an inbred stock albino strain which were bred on a vitamin A-deficient diet showed a high incidence of congenital diaphragmatic hernia, usually involving the posterior half of the right leaf of the diaphragm. Control animals of the same strain bred on the same diet supplemented with a small amount of haliver oil diluted in vegetable oil showed a low incidence of this malformation.

Evidence that nutritional deficiency during pregnancy was responsible for the malformation was provided by breeding rats first on the deficient diet, then feeding them a generous supplement of vitamin A and breeding them a second time. In the 9 rats of this experiment with a high incidence of the malformations in the first litters, no instances of hernia were found in the second litters.

Evidence that the deficient factor, the lack of which led to congenital diaphragmatic hernia, was in fact vitamin A was provided by two experiments: (a) Rats bred for 5 generations on the deficient diet with a supplement of haliver oil gave a low incidence of the defect, whereas the 6th generation bred without supplement gave a high incidence compared with controls of the same generation. (b) Crystalline carotene gave partial protection. The fact that it was partial was explained by the low levels of vitamin A stored in the livers of these rats, suggesting inadequate utilization of the carotene.

The tendency to diaphragmatic hernia is a genetic trait, since the defect was produced in only one rat of 111 of a second strain (Long-Evans), or 0.9 per cent as compared with an incidence of 18.9 per cent in the stock strain on the deficient diet. A substrain (D.H.) was developed from rats of the original stock strain which had a higher incidence of the defect than those of the original stock strain.

It has been demonstrated that the expression of a genetic trait may be enhanced or suppressed by means of diet during pregnancy. Evidence has been provided pointing to vitamin A as the specific nutritional factor responsible in the present experiments.

REFERENCES

1. Andersen, D. H. Cystic fibrosis of the pancreas and its relation to celiac disease. Clinical and pathologic study. *Am. J. Dis. Child.*, 1938, 56, 344–399.
2. Wissler, H., and Zollinger, H. U. Die familiäre kongenitale zystische Pankreasfibrose mit Bronchiektasien. *Helvet. paediat. acta*, 1945, 1, suppl. 1, 3–88.
3. Farber, S. Pancreatic function and disease in early life. V. Pathologic changes associated with pancreatic insufficiency in early life. *Arch. Path.*, 1944, 37, 238–250.

4. Andersen, D. H., and Hodges, R. G. Celiac syndrome. V. Genetics of cystic fibrosis of the pancreas with a consideration of etiology. *Am. J. Dis. Child.*, 1946, **72**, 62–80.

5. Wolbach, S. B., and Howe, P. R. Tissue changes following deprivation of fat-soluble A vitamin. *J. Exper. Med.*, 1925, **42**, 753–777.

6. Warkany, J. Manifestations of Prenatal Nutritional Deficiency. In: Harris, R. S., and Thimann, K. V. (eds.) Vitamins and Hormones. Academic Press, New York, 1945, **3**, 73–103.

7. Hale, F. The relation of vitamin A to anophthalmos in pigs. *Am. J. Ophth.*, 1935, **18**, 1087–1093.

8. Hale, F. Relation of maternal vitamin A deficiency to microphthalmia in pigs. *Texas State J. Med.*, 1937, **33**, 228–232.

9. Warkany, J., and Schraffenberger, E. Congenital malformations induced in rats by maternal vitamin A deficiency. I. Defects of the eye. *Arch. Ophth.*, 1946, **35**, 150–169.

10. Mellanby, H. Effect of maternal dietary deficiency of vitamin A on dental tissues in rats. *J. Dent. Research*, 1941, **20**, 489–509.

11. Wolbach, S. B., and Bessey, O. A. Vitamin A deficiency and the nervous system. *Arch. Path.*, 1941, **32**, 689–722.

12. Moore, L. A. Relationship between carotene, blindness due to constriction of the optic nerve, papillary edema and nyctalopia in calves. *J. Nutrition*, 1939, **17**, 443–459.

13. Andersen, D. H. Incidence of congenital diaphragmatic hernia in the young of rats bred on a diet deficient in vitamin A. (Abstract.) *Am. J. Dis. Child.*, 1941, **62**, 888–889.

14. Ebbs, J. H., Tisdall, F. F., and Scott, W. A. The influence of prenatal diet on the mother and child. *J. Nutrition*, 1941, **22**, 515–526.

15. Burke, B. S., Beal, V. A., Kirkwood, S. B., and Stuart, H. C. Nutrition studies during pregnancy. *Am. J. Obst. & Gynec.*, 1943, **46**, 38–52.

16. Smith, C. A. Effects of maternal undernutrition upon the newborn infant in Holland (1944–1945). *J. Pediat.*, 1947, **30**, 229–243.

17. Mason, K. E. Foetal death, prolonged gestation, and difficult parturition in the rat as a result of vitamin A-deficiency. *Am. J. Anat.*, 1935, **57**, 303–349.

18. Sperry, W. M. Lipid excretion. III. Further studies of the quantitative relations of the fecal lipids. *J. Biol. Chem.*, 1926, **68**, 357–383.

DESCRIPTION OF PLATE

PLATE 23

FIG. 1. Complete right diaphragmatic hernia. The posterior half of the right leaf is absent. Portions of liver and of small intestine lie in the right pleural cavity, compressing the right lung.

FIG. 2. Incomplete right diaphragmatic hernia. The hernial sac is a thin membrane and contains a small nodule of liver.

FIG. 3. Pericardial hernia of the diaphragm. The sac is similar to that of the right incomplete hernia.

1

2

3